A clinical, cytogenetic, and molecular study of 40 adults with the Prader-Willi syndrome

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Abstract

A clinical, cytogenetic, and molecular study has been carried out on 40 adults with a firm diagnosis of Prader-Willi syndrome. A cytogenetically detectable deletion was observed in 58% while further subjects had a deletion which was detectable by molecular methods only, giving a total of 76%. Four cases of maternal uniparental disomy (UPD) were all female. Three of them were heterodisomic while the fourth was isodisomic. Two male probands were heterozygous at all loci tested yet did not have UPD. Although methylation studies showed that one of them had a single band using probe PW71, the other one had two bands. Psychiatric studies suggest that females with maternal UPD are indistinguishable psychologically from those with a paternal deletion in 15q11q13.

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First described in 1956,[1] Prader-Willi syndrome (PWS) is the commonest syndromal cause of obesity. In adults the clinical picture includes hypogonadism, short stature, small hands and feet, hypopigmentation, cognitive impairment, and hyperphagia.[2] Obesity is usually the most prominent physical characteristic of PWS probands and is a major factor in the health problems experienced by them. These may include hypertension, cardiovascular compromise, and diabetes mellitus. Other problems include skin picking (which may be sufficiently serious to cause infections), scoliosis, and eye and dental problems. Most affected people have cognitive impairment and IQs between 60 and 90. Some have severe mental retardation, others have IQs within the normal range.[3 4]

Most cases of PWS are sporadic, although occasional affected sib pairs have been described.[5] PWS is associated with a loss of the paternal contribution in 15q11q13, resulting from de novo deletion of the paternally derived chromosome 15[6 7] or from maternal uniparental disomy (UPD).[8] High resolution banding techniques have shown that the majority of probands have a de novo deletion of the proximal long arm of the paternally inherited chromosome 15, and that other chromosomal abnormalities involving proximal chromosome 15 may account for a further 5% of cases.[9] Molecular studies have suggested that most of the remaining cases have maternal UPD.[10]

Angelman syndrome (AS), although clinically completely distinct from PWS, is associated with a similar deletion in 15q11q13, which is always maternally derived,[11] or with paternal UPD.[12] Thus PWS and its "sister" syndrome, Angelman syndrome (AS) have apparently similar loss of material in 15q11q13 derived from opposite parents suggesting that imprinting of one or more genes within the region is of importance in their expression. Parent specific imprinting results in the differential expression of genes depending on the parent of origin. The oppositely imprinted Prader-Willi critical region (PWSCR) and Angelman syndrome critical region (ASCR) are not allelic but contiguous, with the PWSCR lying proximal to the ASCR.[13] The majority of the deletions detected in both syndromes, however, encompass both critical regions and have similar breakpoints. So, although the two syndromes are distinct, they often share a common deleted region.[13] It has been suggested that hypopigmentation is associated with deletions which include D15S12,[14] but there appears to be no obvious clinical distinction between PWS probands who have a visible 15q11q13 deletion and those who have maternal UPD.[10]

GABRB3 and GABRA5, although both located in the Angelman syndrome region,[15 16] are not considered to be candidate genes for AS,[17] but the location of the SNRPN gene within the critical region for PWS has identified a possible candidate gene which is expressed in the brain, and would exert a pleiotropic effect.[18] Despite this, both PWS and AS are likely to be contiguous gene syndromes.

Because a firm clinical diagnosis of PWS can be difficult in a child and because comparisons of cognitive function are more appropriate in adults, a series of 40 adult probands (age range 16 to 40) with a clinical diagnosis of Prader-Willi syndrome were studied with the following aims: (1) to describe the nature of genetic abnormalities occurring; (2) to characterise the extent and location of deletions within 15q11q13; and (3) to assess the influence, if any, of the nature and size of genetic abnormalities on physical and cognitive characteristics.

Materials and methods

PHYSICAL AND COGNITIVE ASSESSMENT

All of the probands who participated in this study had a diagnosis of Prader-Willi syndrome. To determine whether they fulfilled the diagnostic criteria laid down for PWS they were examined physically and the following information noted: height, weight, standard hand, foot, and eye measurements; testis size in males and menstrual history in females;

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neonatal feeding history and presence or absence of severe hypotonia during the neonatal period; medical and orthopaedic history. Verbal and non-verbal cognitive abilities were assessed using the Raven's Coloured Matrices or Raven's Standard Progressive Matrices (measures of non-verbal cognitive ability)[19 20] and the matching (Crichton or Mill Hill) vocabulary scale (measures of verbal cognitive ability).[21 22] Adaptive behaviour was assessed using the Vineland adaptive behaviour scales.[23] The clinician making the assessments was unaware of the genetic results in all but one case.

CYTOGENETIC STUDIES

Chromosomes were studied by high resolution at the 850 band level. Samples were obtained from the probands, and where possible from both of their parents and any sibs who were available for study. Culture conditions were optimised to obtain prometaphase chromosomes by synchronising cell division with an excess of thymidine or by treatment with ethidium bromide. Harvesting and slide making were by standard methods and the chromosome spreads studied by GTG, RBG, Ag/NOR, distamycin/DAPI, and in some cases by fluorescence in situ hybridisation (FISH). All staining procedures used standard methods and FISH was carried out according to manufacturer's instructions using probes from the critical regions (ONCOR).

MOLECULAR METHODS

DNA was extracted from whole blood using established methods. Conventional Southern blot analysis of RFLPs was carried out using standard techniques. Subjects were genotyped for $(CA)_n$ repeats by PCR amplification of genomic DNA. Briefly, 100 ng genomic DNA was amplified using one ^{32}P-labelled and one unlabelled primer. After amplification, an aliquot of PCR product was analysed by denaturing polyacrylamide gel electrophoresis and the polymorphic bands detected by autoradiography at $-70°C$.

Chromosome 15 probes used for RFLP analysis in 15q11q13 included pML34 (D15S9), pTD3-21 (D15S10), pIR4-3R (D15S11), PIR10-1 (D15S12), and pTD189-1 (D15S13). PCR was carried out using primers detecting microsatellite repeats in the loci D15S11, GABRB3, and GABRA5. Maternal disomy was detected by investigating loci outside the PWSCR. These included D15S24, D15S86, and a $(CA)_n$ repeat within the ACTC (cardiac muscle, alpha actin) gene. Methylation studies at locus D15S63 using probe PW71 were carried out on two probands as described by Dittrich *et al.*[24]

Table 1 Cytogenetic and molecular studies in 40 adult probands with Prader-Willi syndrome

No of probands	*Cytogenetic deletion*	*Molecular deletion by RFLP or PCR*	*Maternal uniparental disomy*
15	+	+	−
6	+	NK, parent missing	
2	+	NI at all loci tested	−
3	−	+	−
4	−	−	+
2	−	−	−
6	−	NK, parent missing	
2	Abnormal karyotype		

NK = Not known. NI = Not informative.

Table 2 Genotypes in three probands with a deletion in 15q11q13 detected by molecular methods only

Subject	*D15S11 RsaI RFLP*	*GABRB3*	*GABRA5*	*D15S24*	*ACTC*	*D15S86*
Proband 1	1.2,(1.2)	1,	2,	b,c	1,2	B,C
Mother	1.2,1.0	1,2	2,4	c,c	1,3	B,C
Father	1.2,1.0	2,2	1,3	a,b	2,4	A,B
Sibs	NK	2,2	NK	a,c	1,4	B,B
Proband 2	3,	3,	3,	a,a	2,3	A,B
Mother	1,3	3,3	3,3	a,b	1,3	A,A
Father	2,4	1,2	1,2	a,a	2,4	B,C
	StyI RFLP					
Proband 3	0.9,	2,	1,	NK	NK	B,C
Mother	0.9, 3.4	2,2	1,3	NK	NK	C,C
Father	2.4 1.9	1,1	2,4	NK	NK	A,B

Results

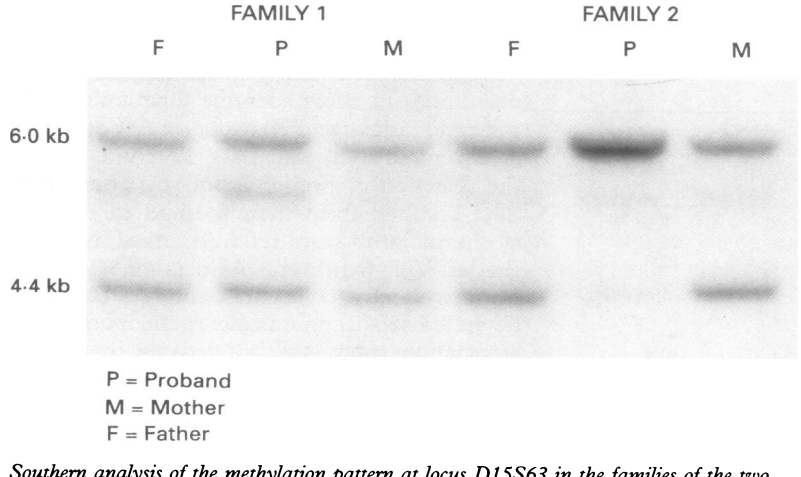
Forty probands with Prader-Willi syndrome and their families participated in the study. All probands had received a diagnosis of PWS from at least one clinician who was not involved in the study and all had a history of neonatal hypotonia and feeding difficulty. A clinical assessment was made of each proband according to criteria laid down[10] and cognitive and behavioural assessments were performed on 20 subjects. Although the accepted diagnostic criteria were used in the assessment of each proband, because the study was aimed at a comparison of physical, behavioural, cytogenetic, and molecular investigations, these criteria could not be used in the differentiation of the probands as having Prader-Willi syndrome or not, as a circular argument would have ensued. Subsequent to cytogenetic and molecular studies they were divided into groups (table 1). In the probands all of the deletions detected were of paternal origin, but none was detected in any of their parents indicating that they were all de novo. A cytogenetically detectable deletion was recorded in 23 (58%) cases, and a further three had a deletion which was only detectable by molecular methods. The smallest region of overlap in all of these cases, however, included not only D15S11, but also the more distal GABRB3 and GABRA5 loci. This region, therefore, includes the minimum region of overlap as defined by Buiting *et al*,[25] and does not represent a subclass of small deletions encompassing the PWSCR only (table 2). Four cases of maternal UPD were detected, three of heterodisomy, and one of isodisomy (table 3). As shown by the detection of loci distal to 15q11q13, in all four cases the disomy pattern was consistent throughout the entire length of the chromosome 15 homologue and a segmental pattern was not observed.

In two male probands a deletion was not detectable by routine cytogenetic methods and heterozygosity was shown at informative loci throughout the PWSCR. Chromosome spreads from each of these two probands were subjected to fluorescence in situ hybridisation (FISH) using probes designated for the PWSCR

Table 3 Genotypes of probands with maternal uniparental disomy

Subject	*D15S9*	*D15S11*	*D15S13*	*D15S10*	*GABRB3*	*GABRA5*	*D15S12*	*D15S24*	*ACTC*	*D15S86*
Proband 1	6.3,6.5	—	2.1,3.5	9,9	1,3	1,3	12,17.5	a,b	—	A,B
Mother	6.3,6.5	—	2.1,3.5	9,9	1,3	1,3	12,17.5	a,b	—	A,B
Father	6.5,6.5	—	2.1,2.1	9,9	1,2	1,2	12,16	c,d	—	A,C
Sib 1	6.5,6.5	—	3.5,2.1	9,9	1,2	1,1	12,16	a,d	—	A,B
Sib 2	6.3,6.5	—	2.1,2.1	9,9	1,3	2,3	12,17.5	—	—	—
Sib 3	6.3,6.5	—	2.1,3.5	9,9	1,2	1,1	12,16	a,d	—	A,B
		StyI/RFLP								
Proband 2	6.3,6.5	0.9,3.4	2.1,3.5	9,9	1,1	3,3	16,17.5	a,b	—	C,D
Mother	6.3,6.5	0.9,3.4	2.1,3.5	9,9	1,1	3,3	16,17.5	a,b	—	C,D
Father	6.5,6.5	0.9,2.4	2.1,3.5	9,9	2,3	1,2	16,16	b,c	—	A,B
Sib	6.3,6.5		3.5,3.5	—	1,2	2,3	16,16	—	—	—
		PCR								
Proband 3	—	2,2	—	—	2,2	—	—	—	2,2	D,D
Mother	—	1,2	—	—	2,2	—	—	—	1,2	A,D
Father	—	3,3	—	—	1,2	—	—	—	3,4	B,C
Proband 4	—	1,3	—	—	1,2	—	—	—	1,3	A,C
Mother	—	1,3	—	—	1,2	—	—	—	1,3	A,C
Father	—	2,3	—	—	1,2	—	—	—	2,4	B,D

(ONCOR) and in each case two signals were detected confirming the absence of a 15q11q13 deletion. When methylation status at locus D15S63 was investigated, one proband carried only the 6.0 kb band, whereas the other one had both the 4.4 kb and the 6.0 kb bands (figure). Two remaining probands each had an abnormal karyotype which involved proximal chromosome 15. One had an inversion with breakpoints at 15p11 and q13, but was informative for the GABRB3 and D15S12 loci. He was not deleted at either locus and had a normal methylation pattern at D15S63 with both the maternal 6.0 kb band and the paternal 4.4 kb band. His mother had a normal karyotype but his father had died so the family could not be investigated further. The second proband had a de novo translocation with the karyotype t(Y;15) (q12;q11.2) which was accompanied by a molecular deletion.[26] Clinical data from a series of male probands are shown in table 4 and from females in table 5. In table 5 clinical data obtained from two female probands with maternal uniparental heterodisomy are compared with data obtained from probands who have a paternal deletion. Comparisons are made both with the total population who have a 15q11q13 deletion and with just the females. The results show that the two females with UPD are physically very similar to the females who have a paternal deletion, and their verbal and non-verbal cognitive scores and their adaptive behaviour scores are also similar, with a tendency to lower verbal adaptive behaviour and higher non-verbal scores. In view of the small number, statistical assessment of these trends was not attempted.



Southern analysis of the methylation pattern at locus D15S63 in the families of the two probands who do not have a paternally derived deletion in 15q11q13 or maternal uniparental disomy. Note the intensity of the 5·5 kb band in the proband from family 1.

Discussion

In this study of 40 adult cases of Prader-Willi syndrome each with a previous firm clinical diagnosis, 23 (58%) probands were found to have a cytogenetically visible deletion in 15q11q13 while a further three had a deletion detectable by molecular methods only, giving a total of 76%. In no case was a deletion detected exclusively in the PWS region, although in two probands a deletion could not be detected by either molecular or cytogenetic methods. Both of these probands were heterozygous for loci within the PWS and AS regions, and both showed two signals of equal intensity when detected by FISH. The absence of the paternally inherited unmethylated 4.4 kb band at D15S63 in one proband implies that there must be either a deletion or maternal uniparental disomy[24] or an imprinting error.[27 28] Maternal UPD was eliminated in both cases, however, after inspection of inheritance patterns at other loci. GABRB3 was informative in both probands. Both males were considered to have classical Prader-Willi syndrome. Recent studies by Glenn *et al*[27] have suggested that in some PWS patients methylation patterns can become disrupted even at loci at which they are heterozygous with an allele inherited from each parent, and imprinting mutations have been implicated in familial cases of both Prader-Willi and Angelman syndromes.[28]

Of two cases with abnormal karyotypes involving proximal chromosome 15q, one de novo 15;Y translocation was shown by molecular methods to have been accompanied by a deletion in 15q11q13,[26] while a case of inversion 15p11p13 could not be further investigated owing to family circumstances. Both probands performed within the range determined for classical PWS.

Only four cases of maternal uniparental disomy were detected among the 40 probands

Table 4 Mean values for stated measures

	Paternal cytogenetic deletion, total population	*Paternal cytogenetic deletion, males only*	*Male with single band at D15S63*	*Male with molecular deletion only*	*Male with molecular deletion only*	*Male with inv 15p11q13*
Height (m)	1.57	1.61	1.63	1.50	1.70	1.55
Weight (kg)	99.9	101.7	100.9	79.5	100.0	92.5
Body mass index (kg m^{-2})	38.8	38.8	38.1	35.3	34.6	38.5
Head circumference (cm)	56.0	57.0	54.0	57.0	55.0	62.0
Middle finger length (cm)	7.3	7.5	7.7	7.6	7.5	7.2
Verbal cognitive ability (y)	8.0	5.5	6.5	5.0	12.0	11.5
Non-verbal cognitive ability (y)	8.0	7.0	6.0	12.0	10.0	11.5
*Adaptive behaviour scores**						
Total	59.0	70.0	62.0	93.0	82.0	67.0
Community living	53.0	60.0	46.0	97.0	70.0	65.0
Daily living	73.0	78.0	95.0	92.0	88.0	66.0
Socialisation	70.0	73.0	62.0	96.0	102.0	86.0

* 20 subjects

tested, three of maternal heterodisomy and one of isodisomy. This represents only 10% of the total. Although there were four further probands who were cytogenetically deletion negative, where one or both parents were unavailable, two of these did not have maternal UPD at locus D15S86, as they both carried an allele which did not come from their mother. For the other two probands, neither parent was available for study, but even if both had UPD this would still represent a total of 15%, considerably lower than the 25 to 30% which has been suggested.[10] Data obtained for two of the patients with maternal heterodisomy suggested that they were indistinguishable both clinically and psychologically from those with a deletion (table 5). The data concerning cognitive ability must be interpreted with caution. The instruments used can be compared by ascertaining the age at which 50% of children attain the score in question. This is helpful to illustrate trends, but does not allow direct comparison in a way which measures generating standard scores do. Despite this, it is still possible to relate these age scores to those in the normal population because results in the matrices and vocabulary scales have a ceiling of 14.5 years, allowing them to be used to compare subjects in terms of age equivalent, although they are not equivalent in the sense that they reflect development which is not necessarily linear. Tables 4 and 5 also incorporate results from the Vineland adaptive behaviour scale which is a standardised instrument with a population mean score of 100 and a standard deviation of 15. Vineland scores are very hard measures of social function and give a social quotient which can be compared in the same way as an IQ score can. The instruments used were chosen as a result of a pilot study and with a view to the time available for assessment. Other studies of cognition have suggested that children and adults with PWS may have an unusual pattern of ability, with a relative weakness in processing information presented aurally and relative strengths in visual perception and organisation.[29–31] There is considerable variation in the overall cognitive impairment associated with PWS. One review found a mean IQ of 57, with a range from 12 to 91.[32] The results presented, however, serve to illustrate trends and to facilitate further analyses. The small numbers preclude more definite comparisons or inferences. The figures are given for females with deletions as all four of the probands ascertained with UPD are female.

The assessments of adaptive behaviour were made with a standardised instrument giving a total score and domain scores which can be compared directly with other populations (mean = 100, standard deviation = 15). The trend towards relatively higher daily living skills and lower community living skills is of interest. The scores are consistent with the view that PWS is associated with mild or borderline handicap. The three males with deletions detectable by molecular methods only had relatively high adaptive behaviour scores. The male with such a deletion and the lowest total score had extremely challenging behaviour and lived in a locked facility which limited his opportunities to show adaptive functioning (the Vineland adaptive behaviour scales measure achievement, not potential). Potential bias in the selection of probands for this study may have resulted from their method of referral, as the majority were referred either from the Prader-Willi Syndrome Association (UK) or from psychiatric colleagues, and may therefore be biased socioeconomically (patient and carer associations may have a relatively small proportion of members from socioeconomic groups 4 and 5) and towards probands with more severe behavioural disorders (those in contact with psychiatric services). Almost any method other than a total population survey is likely to introduce biases, which should be

Table 5 Mean values for stated measures

	Paternal cytogenetic deletion, total	*Paternal cytogenetic deletion, females only*	*Two females with maternal UPD*	*Female with molecular deletion only*
Height (m)	1.57	1.47	1.47	1.50
Weight (kg)	99.9	76.8	78.0	96.4
Body mass index (kg m^{-2})	38.8	38.7	40.3	42.8
Head circumference	56.0	54.0	53.0	—
Middle finger length (cm)	7.3	6.6	6.7	6.8
Palm length (cm)	9.8	9.6	9.3	—
Foot length (cm)	7.4	7.1	6.7	—
Verbal cognitive ability (y)	8.0	9.0	7.0	10.0
Non-verbal cognitive ability (y)	8.0	9.0	10.0	11.0
*Adaptive behaviour score**				
Total	59.0	58.0	46.0	44.0
Community living	53.0	44.0	30.0	40.0
Daily living	73.0	67.0	66.0	54.0
Socialisation	70.0	58.0	55.0	48.0

* 20 subjects

considered when results of surveys are compared.

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