Prader-Willi syndrome in South African patients — clinical and molecular diagnosis

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Study objective. To assess clinically South African patients with the putative diagnosis of Prader-Willi syndrome (PWS) and confirm this diagnosis by DNA/molecular analysis.

Design. Prospective, nationally based, combined clinical and laboratory study.

Main results. Thirty-seven patients with a putative diagnosis of PWS were examined by clinical geneticists. Only 13 (35.1%) of these patients had the diagnosis of PWS confirmed by molecular analysis, and all 13 PWS patients had positive scores using the PWS consensus diagnostic criteria of Holm *et al*. The clinical features of the remaining 24 (64.9%) non-PWS patients were analysed and 23 did not have the neonatal, infantile and childhood features necessary to warrant consideration of a diagnosis of PWS; neither did they obtain a positive score according to Holm *et al*.'s criteria.

Conclusion. PWS was confirmed in only 35% of South African patients with a putative PWS diagnosis, confirming that this condition is overdiagnosed and that the clinical diagnosis is difficult. Clinically, the diagnostic criteria of Holm *et al*. are of great assistance in making the diagnosis, but it remains essential to confirm the diagnosis by molecular analysis.

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Prader-Willi syndrome (PWS), first described in 1956,[1] is a multisystem disorder characterised by infantile hypotonia, obesity, hypogonadism, short stature, mild intellectual disability, behavioural problems, a characteristic facies and small hands and feet.[2-5] Clinical diagnosis of PWS is considered difficult because many of the features are subtle or nonspecific, and others change with age. In

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consequence, underdiagnosis in younger infants and children and overdiagnosis in obese, retarded older children, adolescents and adults occur frequently.[4,5]

The incidence of PWS has been estimated to approximate 1/10 000, but with the recent introduction of molecular diagnostic techniques for PWS, this may be shown to be an overestimation.[2,5] PWS is caused by a deficiency of paternal contributions of the critical region on the long arm of chromosome 15. This can arise from a paternally derived deletion or the presence of maternal uniparental disomy (UPD) of chromosome 15. In a small percentage of cases it is hypothesised that it may be due to an imprinting defect. All of these mechanisms for the causation of PWS are detected by the methylation test using the PW71B probe. PWS has also been described in isolated patients with chromosomal rearrangements of chromosome 15.[4,6-8]

Correct and early diagnosis of PWS is important to ensure relevant counselling and to facilitate appropriate dietary and behavioural management. Furthermore, reversing an incorrect diagnosis of PWS has been recorded to have resulted in considerable parental stress.[5,9] This study was initiated to assess South African patients with the putative diagnosis of PWS and to confirm this diagnosis clinically and by DNA/molecular analysis.

Patients and methods

Patients

The study was initiated in January 1993 and continues to the present. Patients with a clinical diagnosis of PWS were identified from the records of the Prader-Willi Association of South Africa, the Department of Human Genetics at Pretoria University and the Department of Medical Genetics at the University of Cape Town, and from paediatricians in Pretoria, Johannesburg and Durban. Thirty-seven patients with the putative diagnosis of PWS were referred to the participating geneticists in Pretoria (A L C), Cape Town (D V) and Durban (W S W), and their clinical features documented on a protocol derived from Butler.[3] A further 2 patients were seen by another clinical geneticist and a paediatrician with an interest in genetics and their detailed clinical reports were used to obtain the relevant information. On the basis of the clinical information, the patients were scored according to the diagnostic criteria of Holm *et al*. (Table I).[4]

Table I. Clinical diagnostic criteria for Prader-Willi syndrome[4]

Major criteria
1. Neonatal and infantile central hypotonia with poor sucking.
2. Infant feeding problems requiring special feeding techniques and failure to thrive.
3. Excessive weight gain between 12 and 60 months, resulting in central obesity in the absence of intervention.
4. Characteristic facial features in infancy.
5. Hypogonadism.
6. Mild to moderate developmental disability.
7. Hyperphagia/food foraging/obsession with food.

Minor criteria
1. Decreased fetal movement or infantile lethargy or weak cry.
2. Behaviour problems.
3. Sleep disturbance or sleep apnoea.
4. Short stature.
5. Hypopigmentation.
6. Small hands and/or feet.
7. Narrow hands with a straight ulnar border.
8. Eye abnormalities (esotropia/myopia).
9. Thick viscous saliva.
10. Speech articulation defects.
11. Skin picking.

Scoring:
Major criteria are weighted at one point each.
Minor criteria are weighted at half a point each.
Children 36 months of age or younger: five points are required for diagnosis, four of which should come from the major group.
Children 37 months of age to adulthood: total score of eight is necessary for the diagnosis.
Major criteria must comprise five or more points of the total score.

DNA analysis

Molecular analyses of patients and both parents were performed, starting with the methylation test and followed by the use of informative markers to determine the mechanism underlying the PWS.

Methylation status was investigated by Southern blotting and hybridisation of patient DNA with the PW71B probe. This probe detects a parent-of-origin-specific methylation imprint at locus D15S63. The locus is methylated on maternally derived chromosomes and unmethylated on paternally derived chromosomes, and parental DNA samples are therefore not required for the test. If the methylation analysis demonstrates only the maternal pattern, PWS is confirmed.

Informative markers (microsatellite repeats) for loci from within the PWS chromosomal region (D15S11, D15S113, D15S10, GABRB3) and distally outside the region on chromosome 15 (D15S87, CYP19) were used to trace the transmission of chromosome 15 from each parent to the child. This process can identify deletions and UPD by using the markers within the PWS region to detect paternal deletions and the markers outside the PWS region to detect UPD.

These studies were undertaken in the Molecular Genetic Laboratory of the Department of Human Genetics, University of Pretoria, by the fourth and fifth authors.

Cytogenetic analysis

Chromosomal analysis was undertaken in those patients in whom this had not been undertaken previously or in whom a previous chromosomal result could not be obtained. Chromosomal results were thus obtained from five different laboratories in South Africa.

Results

Thirty-seven patients with a putative diagnosis of PWS were clinically assessed and molecular analyses were undertaken (Table II). They comprised 29 (78.4%) Caucasians, 5 (13.5%) patients of mixed ethnic ancestry, 2 (5.4%) black Africans and 1 (2.7%) of Chinese origin. Nineteen (51.4%) individuals were male and 18 (48.6%) were female.

Table II. Clinical, cytogenetic and molecular results of putative PWS patients

Patient No.	Holm score	Chromosome results	DNA results	Final diagnosis
1	6	46,XXdel15q 11-13	Normal	DD/obese
2	9*	46,XY	UPD	PWS
3	8*	46,XX	UPD	PWS
4	5	46,XXdel15q 11-13	Normal	DD/obese
5	3	46,XX	Normal	DD/dysmorphic
6	9*	46,XX	Deletion	PWS
7	7½	46,XY	Normal	Cohen syndrome
8	5	46,1XYdel15 q11-13	Normal	DD
9	8*	46,XY	Deletion	PWS
10	4	46,XX	Normal	DD/obese
11	2	46,XX	Normal	Hypotonia
12	5	46,XY	Normal	DD/short stature/ obese
13	2	46,XX	Normal	DD/dysmorphic
14	10*	46,XY	Deletion	PWS
15	8*	46,XY	Methylation abnormal	PWS
16	8*	46,XX	Methylation abnormal	PWS
17	9½*	46,XX	Deletion	PWS
18	3	46,XY	Normal	DD/obese
19	7½	46,XX	Normal	DD/obese
20	10*	46,XY	Deletion	PWS
21	8*	46,XY	Deletion	PWS
22	8*	46,XY	Normal	DD/obese
23	4	46,XX	Normal	DD/obese
24	4	46,XX	Normal	Obese
25	7½*	46,XY	Deletion	PWS
26	5*	46,XX	Deletion	PWS
27	5½*	46,XY	UPD	PWS
28	3	-	Normal	DD
29	7	-	Normal	DD/obese
30	2½	-	Normal	DD/obese
31	4	46,XY	Normal	DD/overgrowth syndrome
32	6	46,XY	Normal	DD/obese
33	6	46,XYdel15q 11-13	Normal	DD/obese
34	2	46,XX	Normal	DD/obese in infancy
35	3	46,XYdel15q 11-13	Normal	DD/dysmorphic/ obese in infancy
36	5	46,XX	Normal	DD/obese
37	5	46,XX	Normal	DD/seizures/ obese

* Positive Holm *et al.*[4] score.
DD = developmental disability.

On the basis of the clinical information available, all 37 patients were scored according to the diagnostic criteria of Holm *et al.*[4] Fourteen patients (37.8%) had a score equal to, or in excess of, the minimum required for the clinical diagnosis of PWS. The diagnosis of PWS was confirmed by molecular analysis (methylation test) in 13 of the 14 patients. A deletion in the paternally derived chromosome 15 was recorded in 8 (61.5%) and maternal UPD in 3 (23.1%) of the confirmed PWS patients. In 2 patients the mechanism of causation of their PWS could not be ascertained as parental blood was not available for analysis with microsatellites.

Chromosomal results were available for 34 (91.5%) patients. Five of these patients had been reported as having a cytogenetic deletion at 15q(11-13), but on molecular analysis, as well as according to Holm *et al.*'s[4] criteria, none of these patients was confirmed to have PWS. Conversely, of the 8 patients confirmed by molecular analysis to have a paternal deletion on chromosome 15, all had been reported to have normal chromosomes on cytogenetic analysis.

Of the 24 patients in whom the diagnosis of PWS was excluded by molecular analysis, 23 (95.8%) had a negative score according to the criteria of Holm *et al.*[4] One (4.2%) (patient 22, Table II) had a score equal to the minimum required to contemplate the clinical diagnosis of PWS.

The clinical features of the 24 patients in whom a diagnosis of PWS was disclaimed by molecular analysis were analysed in respect of the seven major clinical features of PWS.[4] Twelve (50%) patients had not had neonatal hypotonia, and 16 (66.7%) had not had early feeding problems and failure to thrive, or the classic history of weight gain, or the excessive appetite with foraging for food, characteristic of PWS. In addition, 14 (58.3%) were considered not to have the classic PWS facial features. Eight (72.7%) of the 11 boys had hypogonadism. Obesity was not present in 5 (20.8%) of these patients when assessed by the attending geneticist, but at least 2 had been obese at an earlier stage of their lives. Developmental disability was present in 23 (95.8%) of these 24 individuals.

Collectively, the commonest diagnosis in the non-PWS patients, which was present in 16 (66.7%) of these patients, was developmental disability and obesity with no obvious aetiology. The diagnoses in the remaining non-PWS patients included a single case of Cohen syndrome, developmental disability in a child with an overgrowth syndrome and an obese child with a normal intellect. The remaining 5 patients were all developmentally delayed and in 3 of them a reported chromosomal deletion (15q(11-13)) had contributed significantly to the putative PWS diagnosis (Table II).

Discussion

The characteristics of PWS initially described in 1956 are obesity, short stature, cryptorchidism, mental retardation and lack of muscle tone in infancy.[1] It was not until 1968 that major review articles of PWS emerged, including that of Zellweger and Schneider in which PWS was described as the syndrome of hypotonia-hypomentia-hypogonadism-obesity.[10] This led to the abbreviation HHHO or H_3O to delineate the clinical features of PWS. After these early descriptions of PWS, it became obvious that the clinical features of PWS altered greatly with age; the concept of PWS as a two-stage disorder of infantile hypotonia, feeding problems, failure to thrive (FTT) and developmental delay, followed by a childhood phase in which a voracious appetite and obesity were pre-eminent, emerged. This was later extended to include a third adolescent phase with particular behavioural disorders and medical problems, including diabetes. Recently Donaldson *et al.* suggested that the

model be expanded to include a fourth fetal/neonatal phase.[2,5] The salient features of all four proposed stages are included in the PWS consensus diagnostic criteria of Holm *et al.*,[4] the validity and usefulness of which have subsequently been confirmed.[9,11] In the present study, molecular analysis confirmed that all 13 PWS patients had fulfilled the clinical criteria established by Holm *et al.*[4] There was, however, 1 patient who just satisfied the requirements of these clinical diagnostic criteria,[4] but he had normal chromosomes and, on molecular analysis using the PW71B probe exhibited normal methylation patterns. Given the American Society of Human Genetics and the American College of Medical Genetics recommendations for diagnosis of PWS, this patient is considered not to have PWS.[8] A false-positive clinical diagnosis based on Holm *et al.*'s criteria has been described previously.[4,11]

In this study 8 (61.5%) confirmed PWS patients had a deletion on the paternally derived chromosome 15; 3 (23.1%) patients had maternal UPD and 2 (15.4%) patients were confirmed by methylation studies, using the PW71B probe, alone. This pattern is in keeping with that previously described.[4,6,8,9] False-positive and false-negative cytogenetic results were the rule in this study, with not one correct cytogenetic result being obtained. It has previously been shown that high-resolution chromosome analysis alone is insufficient for deletion detection and that any suspected deletion should be confirmed by molecular techniques.[5,8,9] Conventional cytogenetic analysis should not be used for the definitive diagnosis of PWS but it is still required to be undertaken in suspected cases to diagnose chromosome 15 rearrangements and translocations. This situation prefaces the need for molecular confirmation of the diagnosis of PWS in South Africa. In this study at least 3 patients were incorrectly diagnosed as a result of incorrect cytogenetic findings and, in consequence, their parents were inappropriately counselled. Reversal of a diagnosis of PWS in similar circumstances has caused considerable distress to some of the parents.[5]

The clinical diagnosis of PWS remains difficult as many of the clinical features are nonspecific and age-dependent. Underdiagnosis in younger children and overdiagnosis in obese retarded children and adolescents are common.[4] The latter pattern was confirmed in this study, in which 24 (64.9%) of the 37 putative PWS patients were incorrectly clinically diagnosed. PWS is the commonest syndromic cause of obesity,[6] and 19 (79.2%) of these 24 patients had obesity when seen for this study, while a further 2 (8.3%) had been obese early in their lives. Developmental disability was present in 23 (95.8%) of the non-PWS patients, and it was the combination of these two features, developmental disability and obesity, that led to the putative, albeit incorrect, diagnosis of PWS in 16 (66.7%) of the patients. Further analyses of the clinical features of the 24 non-PWS patients revealed that most did not have the major neonatal, infantile and childhood features necessary to suggest the diagnosis of PWS. Twelve (50%) did not have hypotonia, more than half did not have early feeding problems and FTT (66.7%), or the classic history of weight gain (66.7%) or an obsessive eating pattern, including food foraging (66.7%). Furthermore, 23 (95.8%) did not satisfy the diagnostic criteria of Holm *et al.*[4]

The above figures indicate the need for clinicians dealing with such children to become aware of the natural history and clinical features of PWS, and to utilise the consensus diagnostic criteria of Holm *et al.*[4] to derive a clinical diagnosis. Furthermore, it is now possible to confirm the diagnosis by molecular analysis in South Africa, according to the recommendations of the American Society of Human Genetics and the American College of Medical Genetics.[8]

Finally, it was noted that the diagnosis of PWS was considered in only 2 black patients in this series. PWS is known to occur in all ethnic groups.[2] In neither black patient was the diagnosis confirmed. The authors feel that there is no reason to suspect that the incidence of PWS in the black population should be lower than in the Caucasian population in South Africa. Indeed, as UPD in PWS is associated with advanced maternal age, the incidence theoretically should be higher. However, we would suggest that the prevalence of PWS in the black African population is considerably lower than in the Caucasian population because of early mortality secondary to feeding problems and FTT. An analogous situation, entailing a high incidence, but low prevalence as a result of infant mortality, has recently been described with regard to Down syndrome in the black South African population.[12]

These circumstances, however, highlight the need for the early diagnosis of PWS in the neonatal period. We urge clinicians to include PWS in their differential diagnosis of newborns and infants who present with severe hypotonia and require tube feeding. Donalson *et al.*[5] have also highlighted sticky saliva and a weak/absent cry as significant neonatal diagnostic features. However, it must be reiterated that the final diagnosis of PWS should not be made unless the diagnosis has been confirmed by molecular (DNA) analysis.[8,9]

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