CHROMOSOME 15 IN PRADER-WILLI SYNDROME

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In a previous report (Berry *et al.* 1981) we described three children with features of the Prader-Willi syndrome who had chromosome translocations involving chromosome 15. As a follow-up to that we have karyotyped a selected series of children whose clinical features strongly suggested Prader-Willi syndrome using both good quality Giemsa banding and high-resolution banding techniques. Meanwhile, Ledbetter *et al.* (1981) made a preliminary report of four children with typical Prader-Willi syndrome and a small interstitial deletion of chromosome 15, with break points at 15q11 and 15q13, so that the 15q12 band is deleted. More recently they have reported a larger series with the syndrome, in which this deletion was found in 19 of the 40 patients examined (Ledbetter *et al.* 1982).

Material and method

Patients

Nineteen children ranging in age from two months to nine years were investigated. All were attending the same clinic and were considered to have Prader-Willi syndrome by one observer (V.D.). Their clinical features are summarized in Table I. For a variety of reasons, parental karyotypes were obtained in only three cases.

Chromosome studies

Standard laboratory methods were used to set up cultures and obtain good quality Giemsa-banded preparations. Long chromosomes for high-resolution banding were obtained using modifications of the technique described by Yunis (1981). A methotrexate and bromodeoxyuridine time-sequence was used to synchronise the cell cycle, and actinomycin was added to decrease chromosome contraction.

Microblood cultures were established in medium 199 with 20 per cent DC serum. After 48 hours methotrexate was added (0·05μg/ml final concentration) and left overnight (17 to 19 hours). The block was released by adding BUdR without washing (12μg/ml final concentration) and the culture was incubated for a further six hours. For the last hour actinomycin D3 was added (5μg/ml final concentration), and colcemid (4μg/ml final concentration) for the last 13 minutes. Harvesting was carried out using standard hypotonic potassium chloride for 30 minutes at 37°C and 3:1 methanol:glacial acetic acid fixative. Slide preparations were made using standard air-drying on dry slides from droplets.

Controls

Twenty-nine individuals were chosen at random from those referred to the cytogenetics laboratory, and slides were prepared by routine methods. The 20 best preparations had chromosome 15 examined

TABLE I

Clinical and cytogenetic findings in 19 children with Prader-Willi syndrome

Case no.	*Age yrs:mths*	*Sex*	*Chromosomes*	*Birth hypotonia*	*Swallowing difficulty*	*Undescended testes*	*Typical facies*	*Low IQ*	*Small for gestational age at birth (< 10th centile)*
1	7:0	F	Normal	+	+	–	?	+	–
2	1:6	F	Normal	+	+	–	+	+	+
3	0:6	M	Normal	+	+	+	+	+	+
4	5:0	F	Normal	+	+	–	+	+	–
5	7:0	M	Normal	+	+	+	+	+	+
6	1:6	F	del (15)(q12)	+	+	–	+	+	+
7	8:0	M	del (15)(q12)	+	+	+	+	+	–
8	1:0	F	del (15)(q12)	+	+	–	+	?	+
9	2:6	M	del (15)(q12)	+	+	+	?	+	+
10	1:6	M	del (15)(q12)	+	+	+	?	+	+
11	9:0	M	del (15)(q12)	+	+	+	+	+	+
12	0:1	M	del (15)(q12)	+	+	+	+	?	+
13	0:2	F	del (15)(q12)	+	+	–	+	?	+
14	1:6	F	del (15)(q12)	+	+	–	+	+	+†
15	0:3	M	der (22)(15;22)(q13;q112)**	+	+	+	?	+	+
16	0:8	M	Variant (15)(q12)*	+	+	Retractile	?	+	+
17	2:0	M	Equivocal	+	+	+	?	+	+
18	2:0	M	Detail unreadable	+	+	+	+	+	+
19	0:10	M	Detail unreadable	+	–	?	?	+	+

*Father similar; **father balanced translocation carrier; †born at 32 weeks gestation

Fig. 1. Chromosomes and ideograms to show range of normal variation in q11 to q13 region in long arms of chromosome 15.

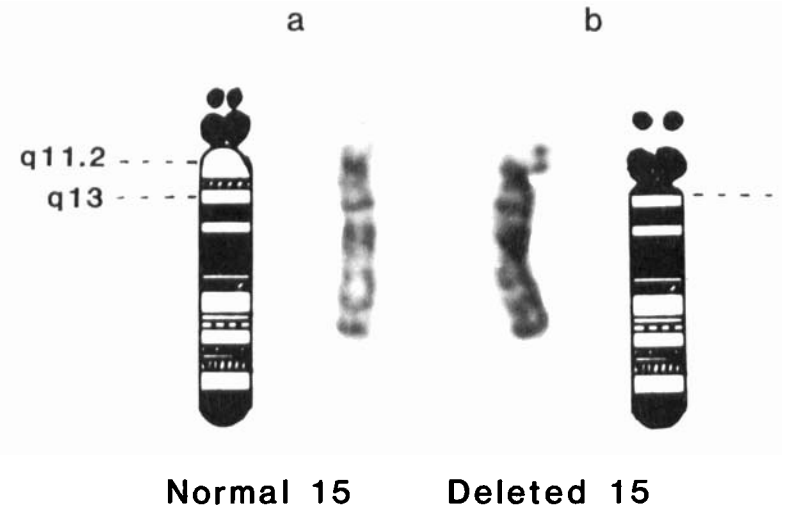

Fig. 2. Ideogram and chromosome showing (a) normal 15 with no heterochromatic variant at centromere; and (b) deleted 15 with heterochromatic variant at centromere.

in 10 to 15 cells, and three from each were photographed. Cells were chosen for length and quality of banding of the chromosomes, so that they were equivalent in detail to those obtained using the high-resolution techniques.

Results

Results are shown in Table I, together with the children's clinical details. Figure 1 shows the range of normal variation on the q11 to q13 region in the long arms of chromosome 15. The deletion of 15q12 illustrated in Figure 2 was present in nine children and the finding was agreed by two independent observers. Five children had a normal chromosome complement, and in two cases the preparations were not good enough for analysis. In one case the findings were equivocal, with the 15q12 deletion being seen in some cells but not in others, possibly due to mosaicism. We were unable to distinguish clinically between cases with the chromosome-15 deletion (Fig. 3, cases 8 and 9) and those with normal chromosomes (Fig. 4, cases 2 and 5).

The remaining two children had other chromosome abnormalities or variants involving chromosome 15. In one there appeared to be a delection of half the 15q12 band and a similar deletion was found in

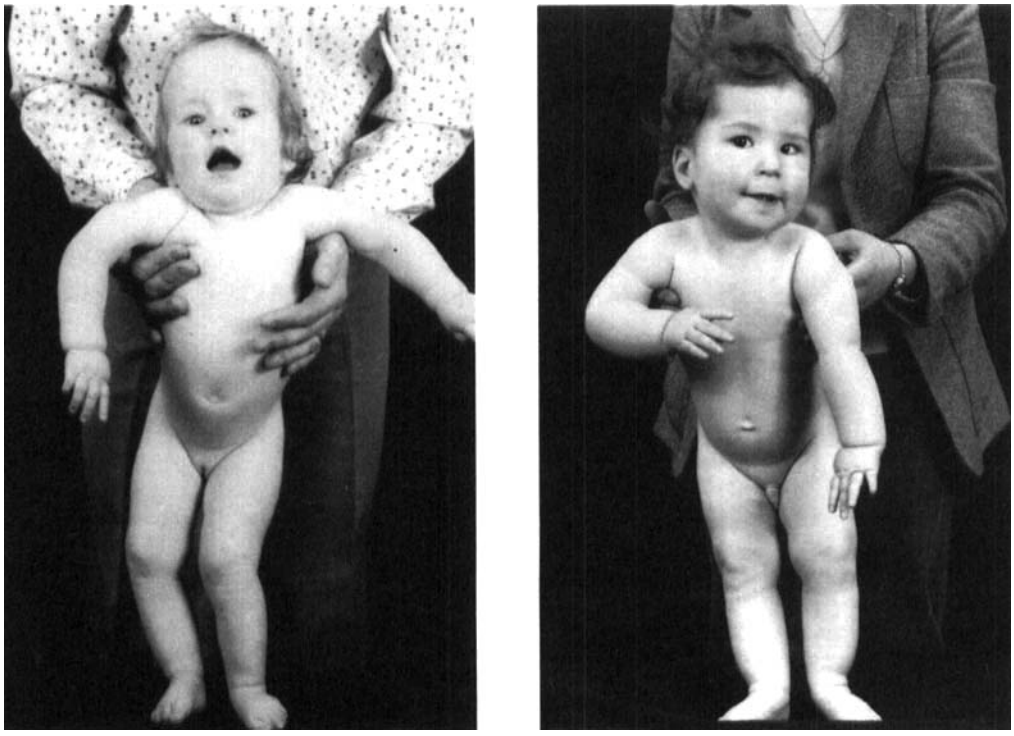

Fig. 3. Female infant (case 8) and male infant (case 9) with classical Prader-Willi syndrome and chromosome-15 deletion.

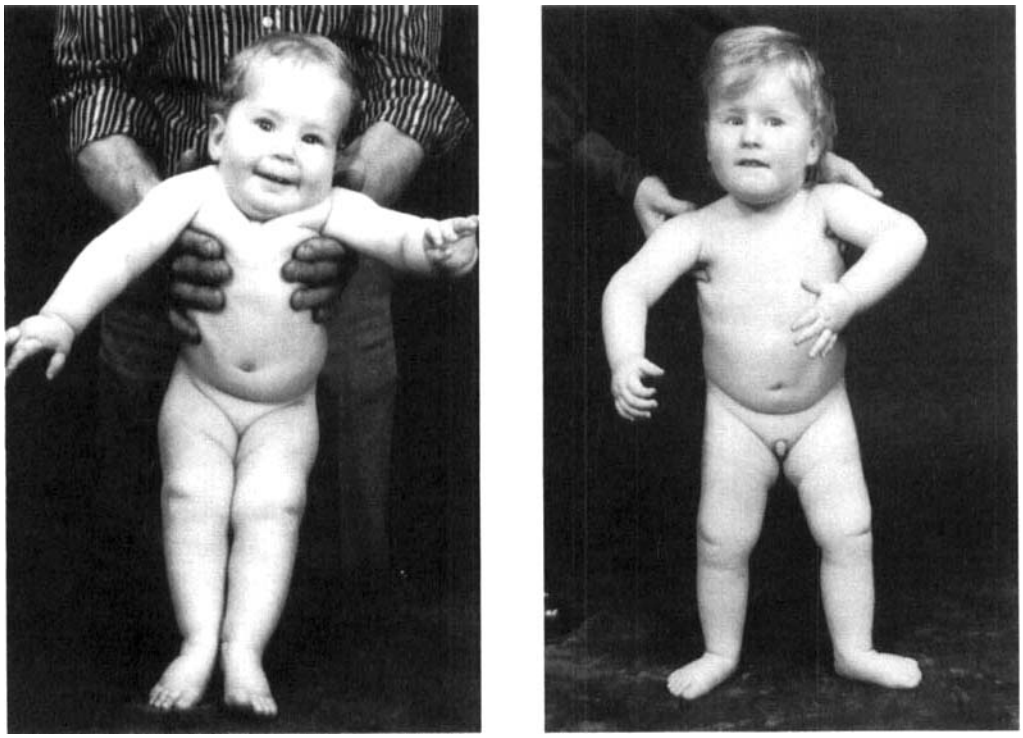

Fig. 4. Female infant (case 2) and male infant (case 5) with classical Prader-Willi syndrome and normal chromosomes.

the father, so this may be a normal variant. The other had an unbalanced reciprocal translocation between chromosome 15 and 22 (chromosome complement 46,XY,-t(15;22) (q13;q112)). The child has chromosome complement 46,XY,der(22)t(15;22) (q13;q112) pat (*i.e.* the translocation is paternal in origin), and is effectively monosomic for 15q12 and the short arm of chromosome 15, and trisomic for the short arm of chromosome 22.

The other parents examined had normal chromosome 15, as did all the controls. It became apparent, however, that the heterochromatic regions of chromosome 15 are extremely variable, and long-arm heterochromatin, when present, may vary considerably in length. 15q12 is a rather lightly staining and narrow band situated very close to the centromere, which makes it particularly difficult to delineate in all but morphologically excellent chromosome preparations. It also varies in size and some individuals have two narrower, darker bands. When present, however, the deletion was frequently visible in preparations from both routine and high-resolution techniques, though high resolution usually was necessary for making the definite decision as to its presence or absence.

Discussion

Our findings show that a recognisable anomaly of chromosome 15 seems to be present in about half the children who have the clinical features of Prader-Willi syndrome. This matches the findings of Ledbetter *et al.* (1982).

Technical problems

The detection of such a small deletion can be difficult and time-consuming. It can only be seen in optimal preparations, and the observer needs to be skilled in high-resolution techniques. There does seem to be considerable normal variation in the region 15q11 to 15q12, so in some families the interpretation of the findings was made easier by comparison with parental karyotypes. Similar difficulties have been described by other workers (Wyandt *et al.* 1981).

The problem of band analysis in this region is emphasised by the brief report by Wulfsberg *et al.* (1981) describing the deletion of the same band in association with Rubinstein-Taybi syndrome, which was later withdrawn and re-interpreted as a normal variation (Wulfsberg *et al.* 1983).

Origin of the abnormal chromosome 15

A further point of interest relating to the parental karyotypes is the origin of the deleted 15. The initial report of Butler and Palmer (1983) described the deleted chromosome as paternal in origin in 11 informative families, and the authors speculated that a coronavirus breakage of chromosome 15 might be responsible. However, more recently Mattei *et al.* (1983) reported two informative cases which were both maternal in origin. Further studies must be awaited to resolve the parental origin before the coronavirus can be seriously considered to be the cause of Prader-Willi syndrome.

Relationship to clinical features

As can be seen from Table I, there is no evident clinical distinction between children with a chromosome abnormality and those without. The children were probably selected to some extent in that they were attending a clinic with considerable interest in muscle disorders, so hypotonia is likely to be a constant feature.

Mattei *et al.* (1983) claimed that there is a clinical distinction between individuals diagnosed as Prader-Willi and later found to have the 15q deletion, and those with normal chromosomes. Their cases with normal chromosomes had no feeding difficulties and were not all hypotonic, so they do not really have sufficient criteria to fit the Prader-Willi diagnosis. Therefore one might question the grounds for their inclusion in the study. We could not make any clinical distinction between our cases who turned out to have normal chromosomes and those who had the deletion, and would expect those who have normal chromosomes to have some form of deletion at the genic level which is not resolvable at the chromosomal level.

Another paper has recently confirmed the association between the syndrome and abnormalities of chromosome 15. In the multicentre study of Fraccaro *et al.* (1983), the clinical features of Prader-Willi were present in individuals with a variety of

chromosome abnormalities, all of which involved chromosome 15 (two balanced reciprocal translocations, three unbalanced, one unidentified marker and one with the interstitial deletion of 15q). The clinical diagnosis of Prader-Willi syndrome was sometimes made before the cytogenetic diagnosis was known, but in others it was made after.

Cytogenetic findings

Chromosome anomalies in Prader-Willi syndrome have been reported sporadically since 1963, and chromosome 15 is implicated with striking frequency, yet it is difficult to discern any distinct pattern linking all the reported abnormalities.

There are now more than 40 reported cases of deletion (15) (q12). A review by Kousseff (1982) lists nine patients with 15/15 Robertsonian translocations, 15 with translocations between 15 and another autosome, and five with *additional* chromosomal material derived from the short arms and proximal part of the long arms of chromosome 15. With this great variability in abnormality, Kousseff asks whether the chromosome abnormality may possibly be a secondary feature of Prader-Willi syndrome rather than its origin.

Robertsonian translocations could give rise to effective monosomy for 15 (q11;q12) if there was gene inactivation due to chromosome rearrangement around the centromere, but this explanation could not be applied to those cases in which there appeared to be additional 15 short-arms.

Thus the situation appears to be more complicated than that for other chromosome-deletion syndromes. The association of retinoblastoma with deletion of the long arm of chromosome 13 is well recognized, as is that between aniridia and Wilms tumour, with short-arm deletion of chromosome 11 (Riccardi *et al.* 1978). Several cases of Langer-Giedion syndrome with long-arm deletion of chromosome 8 have now been described (Turleau *et al.* 1982) and very recently deletion of the short arm of 17 has been found in two children with the Miller-Dieker syndrome (Dobyns *et al.* 1983). Clearly we are entering a new era in syndromology.

Conclusions

The association of Prader-Willi syndrome with structural anomaly involving chromosome 15 seems to be established. However, as these anomalies are not always present in classically affected children, and as the distinction between true abnormality and normal variation can be subtle, interpretation of the abnormality is difficult. Further developments in this interesting field must be awaited to resolve some of the outstanding questions.

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SUMMARY

Nineteen children with the clinical features of Prader-Willi syndrome were karyotyped, using both routine Giemsa banding and high-resolution techniques. Chromosome abnormalities involving chromosome 15 were found in 10, entirely normal chromosomes in five and for the remaining four the findings were either equivocal or difficult to interpret. There was no clinical distinction between cases with and without the chromosome anomaly. Examination of three parents and a group of controls showed that the proximal end of the long arm of chromosome 15 may have a considerable degree of normal variation, which can make interpretation difficult.

RÉSUMÉ

Chromosome 15 et syndrome de Prader-Willi

Un caryotype, utilisant les techniques de routine de bandes de Giemsa et de haute résolution, a été pratiqué chez 19 enfants présentant les caractéristiques cliniques du syndrome de Prader-Willi. Des anomalies chromosomiques impliquant le chromosome 15 ont été trouvées dans 10 cas, les chromosomes étaient entièrement normaux dans cinq cas, les données étaient équivoques ou difficiles à interpréter dans les quatre cas restants. Il n'y avait pas de distinction clinique entre les cas avec ou sans les anomalies chromosomiques.

L'examen de la parenté et des contrôles a montré que l'extrémité proximale du bras long du chromosome 15 peut présenter un degré considérable de variation normale, ce qui peut rendre l'interprétation difficile.

ZUSAMMENFASSUNG

Chromosom 15 beim Prader-Willi Syndrom

Von 19 Kindern mit den klinischen Merkmalen des Prader-Willi syndroms wurde durch die Giemsa Bandentechnik und durch hohe Auflösungstechniken der Karyotyp bestimmt. Bei 10 Kindern wurden Veränderungen des Chromosoms 15 gefunden, fünf hatten ganz normale Chromosomen und bei den restlichen vier waren die Befunde unklar oder schwer zu interpretieren. Klinisch fand sich kein Unterschied zwischen den Fällen mit und ohne Chromosomenveränderung. Untersuchungen von Eltern und Kontrollen haben gezeigt, daß das proximale Ende des langen Armes beim Chromosom 15 viele Normvarianten hat, wodurch die Interpretation erschwert werden kann.

RESUMEN

El cromosoma 15 en el síndrome de Prader-Willi

Se determinó el cariotipo de diecinueve niños con las características clínicas del sindrome de Prader-Willi, utilizando las técnicas de las bandas de Giemsa y de alta resolución. En 10 casos se hallaron anomalías en el cromosoma 15 y cromosomas normales en cinco, mientras que los otros cuatro casos era de interpretación equívoca o dificil. No había ninguna diferencia clínica entre los casos con o sin la anomalia cromosómica. El examen de los padres y de los controles mostró que el extremo proximal del brazo largo del cromosoma 15 muestra un grado considerable de variación normal, lo cual puede dificultar la interpretación.

References

Berry, A. C., Whittingham, A. J., Neville, B. G. R. (1981) 'Chromosome 15 in floppy infants.' *Archives of Disease in Childhood,* **56,** 882–885.

Butler, M. G., Palmer, C. G. (1983) 'Parental origin of chromosome 15 deletion in Prader-Willi syndrome.' *Lancet,* **1,** 1285–1286.

Dobyns, W. B., Stratton, R. F., Parke, J. T., Greenberg, F., Nussbaum, R. L., Ledbetter, D. H. (1983) 'Miller-Dieker syndrome: lissencephaly and monosomy 17p.' *Journal of Pediatrics,* **102,** 552–558.

Fraccaro, M., Zuffardi, O., Bühler, E., Schinzel, A., Simoni, G., Witkowski, R., Bonifaci, E., Caufin, D., Cignacco, G., Delendi, N., Gargantini, L., Losanowa, T., Marca, L., Ullrich, E., Vigi, V. (1983) 'Deficiency, transposition, and duplication of one 15q region may be alternatively associated with Prader-Willi (or a similar) syndrome. Analysis of seven cases after varying ascertainment.' *Human Genetics,* **64,** 388–394.

Kousseff, B. G. (1982) 'The cytogenetic controversy in the Prader-Labhart-Willi syndrome.' *American Journal of Medical Genetics,* **13,** 431–440.

Ledbetter, D. H., Mascarello, J. T., Riccardi, V. M., Harper, V. D., Airhart, S. D., Strobel, R. J. (1982) 'Chromosome 15 abnormalities and the Prader-Willi syndrome: a follow-up report of 40 cases.' *American Journal of Human Genetics,* **34,** 278–285.

— Riccardi, V. M., Airhart, S. D., Strobel, R. J., Keenan, B. S., Crawford, J. D. (1981) 'Deletions of chromosome 15 as a cause of the Prader-Willi syndrome.' *New England Journal of Medicine,* **304,** 325–328.

Mattei, J. F., Mattei, M. G., Giraud, F. (1983) 'Prader-Willi syndrome and chromosome 15.' *Human Genetics,* **64,** 356–362.

Riccardi, V. M., Sujansky, E., Smith, A. C., Francke, U. (1978) 'Chromosomal imbalance in the aniridia—Wilms tumour association: 11p interstitial deletion.' *Pediatrics,* **61,** 604–610.

Turleau, C., Chavin-Colin, F., de Grouchy, J., Maroteaux, P., Rivera, H. (1982) 'Langer-Giedion syndrome with and without del 8q. Assignment of critical segment to 8q23.' *Human Genetics,* **62,** 183–187.

Wulfsberg, E. A., Klisak, I. J., Sparkes, R. S. (1981) 'Chromosome 15q interstitial delections in Rubinstein-Taybi syndrome.' *American Journal of Human Genetics,* **33,** 127A (*Abstract no. 383*).

— — — (1983) 'High resolution chromosome banding in the Rubinstein-Taybi syndrome.' *Clinical Genetics,* **23,** 35–37.

Wyandt, H. E., Patil, S., Shah, H. O., Hanson, J. W., Zellweger, H., Kelly, T. E., Dolan, L. M., Wilson, W. G. (1981) 'Problems in the detection of 15q deletion in patients with Prader-Willi syndrome.' *American Journal of Human Genetics,* **33,** 127A (*Abstract no. 384*).

Yunis, J. J. (1981) 'Mid-prophase human chromosomes. The attainment of 2000 bands.' *Human Genetics,* **56,** 293–298.