Hum Genet (1992) 88:376–378

Human Genetics



*Original investigations*

# Maternal origin of deletion 15q11–13 in 25/25 cases of Angelman syndrome

**J. Clayton Smith[1], T. Webb[2], M. E. Pembrey[1], M. Nichols[1], and S. Malcolm[1]**

[1]Mothercare Department of Paediatric Genetics, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK
[2]Department of Clinical Genetics, Birmingham Maternity Hospital, Birmingham, UK

Received April 2, 1991 / Revised June 13, 1991

**Summary.** About half of the cases of Angelman syndrome arise from deletions of chromosome band 15q12. In 25 cases we have been able to determine the parental origin of the deletion and, in line with other reported cases, we have found the deletion to be of maternal origin. There were no exceptions. The parental origin was determined using cytogenetic markers in 13 of the cases, in nine by using the pattern of inheritance of restriction fragment length polymorphisms, and in three using both techniques.

## Introduction

Some of the most compelling evidence for genomic imprinting in man is emerging from the study of Angelman and Prader-Willi syndromes. Although they have quite different clinical phenotypes (Butler 1990; Angelman 1965) they can both arise from deletions of chromosome band 15q12 (Pembrey et al. 1989; Magenis et al. 1987). Molecular genetic studies have so far failed to distinguish between the deletions in the two diseases (Donlon 1988; Nicholls et al. 1989a; Knoll et al. 1989, 1990), except in respect of their parental origins. Prader-Willi deletions arise on the chromosome inherited from the father (Butler and Palmer 1983) and Angelman syndrome on the chromosome inherited from the mother (Knoll et al. 1989; Magenis et al. 1990).

We report here data from a large series of Angelman patients and confirm the exclusively maternal origin of the de novo deletions. Two methods were used to study parental origin, cytogenetic and molecular genetic. Chromosome 15 is polymorphic with respect to the size of the short arm, the presence or absence of satellites and the activity of the nucleolus organiser regions as demonstrated by silver staining. These variations often make it possible to distinguish the individual chromosome 15s and to follow their inheritance within the family. A number of DNA probes from the critical region which identify RFLPs have been isolated (Nicholls et al. 1989a). In some cases the inheritance of alleles of the polymorphism shows that there is a deletion of one copy of that sequence in the child and reveals the parental origin. However, many cases are non-informative.

*Offprint requests to:* S. Malcolm

## Materials and methods

### *Patients*

Patients were referred from a number of centres and the diagnosis of Angelman syndrome was confirmed in each case on the basis of the clinical history, examination and EEG findings. All patients had delayed motor milestones, absent speech, ataxia and facial features typical of the syndrome, including microbrachycephaly, wide mouth, prominent lower jaw and a tendency to tongue protrusion. Twenty four of the 25 patients were seen by one of the authors.

### *Cytogenetic analysis*

Elongated prometaphase chromosomes at the 850 band level were obtained by setting up at least eight cultures per individual and by employing thymidine blocks with release at different time intervals or, alternatively, by the addition of ethidium bromide to cultures in two different media.

Harvesting and slide making were by routine methods, but slides were subjected to both Giemsa-banding and silver staining for the demonstration of NORs (Bloom and Goodpasture 1976).

### *Molecular genetic analysis*

*Probes.* DNA probes IR10-1 (D15S12), p34 (D15S9), 189-1 (D15S13), 3-21 (D15S10) and IR4-3R (D15S11) were used for RFLP analysis (Nicholls 1989a). Polymorphic alleles were revealed by *ScaI* in the first two cases, by *TaqI* in the second two, and by *RsaI* in the last.

*Restriction enzyme analysis.* DNA was extracted from blood collected into EDTA tubes by guanidinium hydrochloride and Proteinase K extraction (Jeanpierre 1987). Agarose gels were run in standard fashion and, following denaturation, the DNA was blotted directly in alkali onto Hybond N+. DNA was fixed to the filter by baking at 80°C. The DNA probes were radiolabelled using random hexanucleotide priming. Following hybridisation the filters

were washed in 3 × SSC, 0.1% SDS at room temperature for 1h and 1 × SSC, 0.1% SDS at 65°C for 20 min, and exposed using Kodak XOMAT film.

## Results

DNA analysis was useful in determining the parental origin of the deletion in 11 cases. Eight of these had a visible cytogenetic deletion of band 15q12 (Fig. 1). High resolution cytogenetic studies had not been undertaken in three cases.

The results are presented in Table 1. Family DS illustrates the method. For probe IR10-1 (D15S12) the affected child had only the 16.1 kb allele and his mother only the 12.5 kb allele. Therefore, there had been a de novo deletion of this stretch of DNA.

Cytogenetic analysis revealed the origin of the mutations in 7 cases. The microscopic appearance of the short arms of chromosome 15 was recorded and silver staining was carried out where fresh preparations were available. The categories used to describe the variations in satellite size were S1 (small), S2 (medium) and S3 (large). Additionally p is used to describe a chromosome 15 with a short arm but no satellite, and S0 to describe a chromosome 15 with neither visible short arms nor satellites. The results are presented in Table 2.

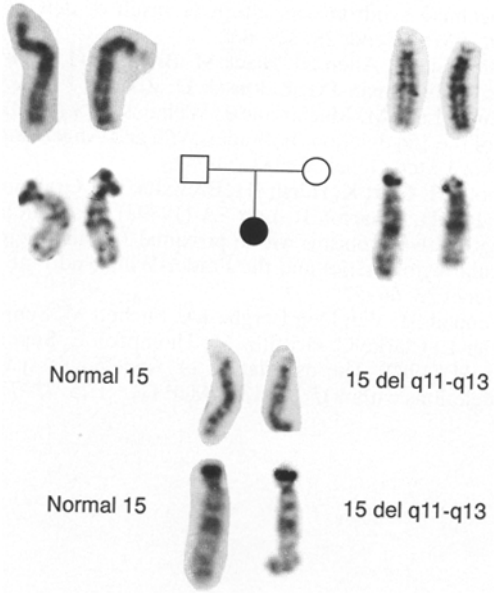


**Fig. 1.** Chromosome deletion of 15q11–13 in family MN and the pattern of inheritance of silver staining revealing maternal deletion. The affected child, a girl, has a 15q11–13 deletion (RHS) as shown by GTG-banding. As all four parental chromosomes 15 are satellited it is not possible to ascertain whether the deleted chromosome was inherited from her mother or father. Silver staining revealed that the 15 del q11–q13 chromosome is maternally inherited as the normal paternally inherited chromosome has an Ag2 level of staining at the tip of the satellite whereas the deleted chromosome has Ag2 staining across the centre of the satellites, exactly as in one of the maternal chromosomes 15

**Table 1.** Alleles with probes from the 15q11–13 region. M, Mother; F, father; C, child

| Family | Probe | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1R10-1 (D15S12) | | | 189-1 (D15S13) | | | 1R4-3R (D15S11) | | | 3-21 (D15S10) | | |
| | M | F | C | M | F | C | M | F | C | M | F | C |
| *Families with cytogenetic deletion* | | | | | | | | | | | | |
| DS | 3 | 2 | 2 | | | | | | | | | |
| GES | | | | 2 | 1 | 1 | | | | | | |
| HIS[a] | 2 | 1,2 | 1 | | | | | | | | | |
| SD | | | | | | | | | | 1 | 1,2 | 2 |
| WHD | | | | | | | | | | 1 | 1,2 | 2 |
| BM | | | | | | | | | | 1 | 1,2 | 2 |
| WOD[a] | | | | 1 | 1,2 | 2 | | | | | | |
| WN | | | | | | | 1 | 1,2 | 2 | | | |
| *No high resolution banding available* | | | | | | | | | | | | |
| BT | 2 | 2,3 | 3 | | | | | | | | | |
| RAE | | | | | | | | | | 2 | 1 | 1 |
| ON | | | | 1 | 1,2 | 2 | | | | 1 | 1 | 1 |

[a] Family also in Table 2

**Table 2.** Cytogenetic deletions. S1, Small satellite; S2, medium satellite; S3, large satellite; p, short arm with no satellite; S0, no short arm or satellite; Ag0, no silver staining; Ag1, small dots of silver; Ag2, medium sized dots; Ag3, heavy silver staining

| Family | Mother | | Father | | Child | |
|---|---|---|---|---|---|---|
| | | | | | Deleted chromosome 15 | Normal 15 |
| *Short arm markers* | | | | | | |
| TY | S1 | S1 | S2 | S2 | S1 | S2 |
| HK | S2 | S2 | S1 | S2 | S2 | S1 |
| GZI | NA | NA | S2 | S2 | p | S2 |
| DK[a] | S1 | S2 | S2 | S2 | S1 | S2 |
| WOD[b] | S3 | S2 | p | S2 | S3 | S2 |
| CN | S1 | S2 | S1 | S3 | S1 | S3 |
| LN | S1 | S2 | NA | NA | S1 | p |
| HIS[b] | S0 | S1 | S2 | p | S0 | S2 |
| GDI | S0 | S2 | S1 | S2 | S0 | S2 |
| MR | S1 | S2 | S2 | p | S1 | p |
| CE | S3 | S3 | NA | NA | S3 | S1 |
| SN | p | S1 | S2 | S2 | p | S2 |
| RUE | S1 | S1 | S1 | p | S1 | p |
| HT | p | S2 | S2 | S2 | p | S2 |
| *Silver staining* | | | | | | |
| MN | Ag1 | Ag2 | Ag2 tip | Ag2 tip | Ag2 | Ag2 tip |
| JE | Ag1 | Ag2 | Ag0 | Ag2 | Ag2 | Ag0 |

[a] Maternal deletion demonstrated with D15S9 and D15S10 (J. Knoll personal communication)
[b] Family also in Table 1

The silver staining was reported in terms of intensity (Ag0–Ag3) of distinguishable dots of stain and the position of the silver on the satellite stalks (Fig. 1).

In two families the fathers were not available for analysis and in one case the mother was not available. However, in the first two cases it was possible to identify a non-deleted chromosome in the child; this was clearly not of maternal origin. For example, in family CE both the maternal chromosome 15s had large satellites (S3) but the child had a non-deleted chromosome 15 with small satellites (S1) in addition to a 15q11–13 deletion on a chromosome with large satellites. The small satellited chromosome must have been the chromosome inherited from father. A further three cases were not fully informative but were all consistent with maternal inheritance (data not shown).

## Discussion

Prader-Willi syndrome has been well recognised and described for a number of years (Butler 1990). The presence of a cytogenetic deletion, 15q11–13, has been well established and its paternal origin noted. Angelman syndrome has been recognised and reported less often (Angelman 1965) but is increasingly being diagnosed. An association with a cytogenetic 15q11–13 deletion is evident here too (Pembrey et al. 1989; Magenis et al. 1987) although its exact frequency has not yet been determined.

The cytogenetic study by Magenis (1990) suggested that the deletions found in Angelman syndrome, although involving the same region as in Prader-Willi, were larger in that they tended to remove more of the light band 15q13. However, the limited number of probes available up to now have failed to reveal obvious differences in the position or extent of the deletions. The startling difference is in parental origin: in all 14 reported cases the deletions in Angelmans syndrome have arisen on the chromosome of maternal origin (Magenis et al. 1990; Knoll et al. 1989; Williams et al. 1990). We have now extended the series with an additional 25 cases and have found no exceptions. On preliminary cytogenetic analysis, two cases, HIS and DS, were reported previously as being of paternal origin (Malcolm et al. 1990). We have now shown HIS to be of maternal origin by both cytogenetic and molecular genetic analysis and, using probe IR10-1, DS is also a clear deletion of maternal origin.

*Acknowledgements.* This work was supported by Action Research for the Crippled Child and the Child Health Research Appeal Trust. We gratefully thank Joan Knoll for unpublished information on patient DK.

## References

Angelman H (1965) "Puppet" children: a report on three cases. Dev Med Child Neurol 7:681–683

Bloom SE, Goodpasture C (1976) An improved technique for selective silver staining of nucleolar organiser regions in human chromosomes. Hum Genet 34:199–206

Butler MG (1990) Prader-Willi syndrome: current understanding of cause and diagnosis. Am J Med Genet 35:319–332

Butler MG, Palmer CG (1983) Parental origin of chromosome 15 deletion in Prader-Willi syndrome. Lancet I:1285–1286

Donlon TA (1988) Similar molecular deletions on chromosome 15q11.2 are encountered in both Prader-Willi and Angelman syndrome. Hum Genet 80:322–328

Jeanpierre M (1987) A rapid method of purification of DNA from blood. Nucleic Acids Res 15:9611

Knoll JHM, Nicholls RD, Magenis RE, Graham JM, Lalande M, Latt SA (1989) Angelman and Prader-Willi syndromes share a common chromosome 15 deletion but differ in parental origin of the deletion. Am J Med Genet 32:285–290

Knoll JHM, Nicholls RD, Magenis RE, Glatt K, Graham J, Kaplan L, Lalande M (1990) Angelman syndrome: three molecular classes identified with chromosome 15q11q13 specific DNA markers. Am J Hum Genet 47:149–155

Magenis RE, Brown MG, Lacy DA, Budden S, Le Franchi S (1987) Is Angelman syndrome an alternate result of del(15)(q11q13)? Am J Med Genet 28:829–838

Magenis RE, Toth-Fejel S, Allen LJ, Black M, Brown MG, Budden S, Cohen R, Friedman JM, Kalousek D, Zonana J, Lacy D, La Franchi S, Lahr M, Macfarlane J, Williams CPS (1990) Comparison of the 15q deletions in Prader-Willi and Angelman syndromes. Am J Med Genet 35:333–349

Nicholls RD, Knoll JH, Glatt K, Hersh JH, Brewster TD, Graham JM, Wurster-Hill D, Wharton R, Latt SA (1989a) Restriction fragment length polymorphisms within proximal 15q and their use in molecular cytogenetics and the Prader-Willi syndrome. Am J Med Genet 33:66–77

Pembrey ME, Fennell SJ, Van Den Berghe JA, Fitchett M, Summers D, Butler L, Clarke C, Griffiths M, Thompson E, Super M, Baraitser M (1989) The association of Angelman's syndrome with deletions within 15q11–13. J Med Genet 26:73–77