Clinical features in 27 patients with Angelman syndrome resulting from DNA deletion

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Abstract
We report the clinical features in 27 Australasian patients with Angelman syndrome (AS), all with a DNA deletion involving chromosome 15(q11-13), spanning markers from D15S9 to D15S12, about 3-5 Mb of DNA. There were nine males and 18 females. All cases were sporadic. The mean age at last review (end of 1994) was 11-2 years (range 3 to 34 years). All patients were ataxic, severely retarded, and lacking recognisable speech. In all patients, head circumference (HC) at birth was normal but skewd in distribution, with 62-5% at the 10th centile. At last review HC was around the 50th centile in three patients (12-5%) while 15 had poor postnatal head growth. Short stature was not invariable, 5/26 (19%) were on or above the 50th centile. Hypotonia at birth was recorded in 15/24 (63%) and neonatal feeding difficulties were recorded in 20/26 (77%). Epilepsy was present in 26/27 (96%) with onset by the third year of life in 20 patients (83%). Improvement in epilepsy was reported in 11/16 patients (69%) with age. An abnormal EEG was reported in 25/25 patients. Hypopigmentation was present in 19/26 (73%). One patient had oculocutaneous albinism. Five patients could not walk independently. Of the remaining 22 who could walk, age of onset of walking ranged from 2 to 8 years. Disrupted sleep patterns were present in 18/21 patients (86%), with improvement in 9/12 patients (75%) over 10 years of age.

The clinical features in this group of deletional AS patients were similar to previous reports, but these have not separated patients into subgroups based on DNA studies. In our group of deletional cases, 100% showed severe mental retardation, ataxic movements, absent language, abnormal EEG, happy disposition (noted in infancy in 95%), normal birth weight and head circumference at birth, and a large, wide mouth. These features occurred with a higher frequency than in AS patients as a whole. Our study also provided information on the evolution of the phenotype. The data can act as a benchmark for comparisons of AS resulting from other genetic mechanisms.

Angelman syndrome (AS) is an intellectual disability disorder with a complex genetic etiology. Since the first three children reported 30 years ago,1 the clinical phenotype has been described in many individual cases,2-4 from patient surveys,5-7 and reviews of published reports.141213 The phenotype of AS comprises severe intellectual disability, epilepsy, lack of speech, ataxic movements, easily precipitated laughter, fair colouring in some patients, large mouth and chin, microcephaly, and an abnormal EEG. Phenotypic variability has been noted and some cases have been described as atypical.111415 The lack of a distinctive early phenotype is reflected by the age of diagnosis in the majority of patients described, ranging from 2 years6 to adulthood.16

The availability of sophisticated genetic studies, including high resolution cytogenetics, fluorescence in situ hybridisation (FISH), and DNA testing by methylation assays or DNA polymorphic markers17-21 has enabled a genetic classification of AS. Interstitial deletions of chromosome 15(q11-13) comprise about 66% of cases, and non-deletional AS about 33% of cases.2 In the latter group about 2% are the result of uniparental disomy (UPD)182122 and a few cases have been described with an imprinting mutation.23 The DNA diagnosis is highly specific as the AS locus is subject to imprinting so that both deletions and UPD show a parent of origin effect, with the deletions maternally in origin24 and UPD paternal.2122 The DNA deletion in AS may be quite small23 but a large deletion spanning about 3-5 Mb of DNA from D15S9 proximally to D15S12 distally is seen in over 90% of deletional cases.24

Most reported clinical studies have either not used DNA diagnosis or have considered all cases together. Other studies have used high resolution cytogenetics to classify patients as deleted or non-deleted.17-25 High resolution cytogenetics has been shown to be inaccurate.26-28 There are now a number of reports describing the DNA types in AS patients,22-31 but these have not included clinical features.

Laboratory confirmation of the diagnosis of AS has now made it possible to assess clinical features in relation to the DNA based genetic classification. The phenotypic heterogeneity described in AS may in part be attributable to differences in the underlying genetic mechanisms. Such evaluations would also provide specific information useful for diagnosis and prognosis. One recent study has reported 61 patients with AS (37 with sporadic deletion) and examined several clinical features in de-
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Methods

PATIENTS

Patients were referred from Australia and New Zealand for genetic testing under a research grant protocol, approved by the institutional Ethics Committee. A data sheet accompanied each referral. The data sheet contained questions on AS based on the American AS Association checklist. Twenty-two cases were seen by the senior author (AS) as well as the referring doctor, while information on five cases (8, 10, 19, 23, 24) was obtained only from the data sheets and correspondence with the referring geneticist. Consecutive cases (1991–1994) with a maternal deletion, extending from D15S9 to D15S12, were included in this study. Clinical assessment of all patients was made before the DNA results were available. Further information was sought from hospital records, referring physicians, other physicians involved with the patient care, Baby Health Centre records, parent interviews, early photographs, and videos. All patients/records were reviewed at the end of 1994.

GENETIC TESTING

Cytogenetic analysis was performed on all probands, on peripheral blood lymphocytes, harvested after 72 hour cultures and karyotyped after GTG banding had been performed. Molecular studies were performed on the parents and both parents in 26/27 cases. High molecular weight DNA was prepared from peripheral blood according to standard methods. Polymorphism analysis was performed with RFLP, VNTR, and PCR based simple sequence repeats (SSR) with probes for loci from within the PWS/AS chromosome region (D15S18, D15S9, D15S11, D15S13, D15S10, D15S113, D15S97, GABBR3, D15S12) and outside the region distally on chromosome 15 (D15S24, ACTC, THBS1, D15S87, D15S86). DNA patterns at each polymorphic locus were recorded according to the bands seen on the autoradiographs or polyacrylamide gels. Detailed methods and the probe–enzyme combinations used have been previously described for patients AS1–10 and subsequent patients were investigated in the same way. Usually five polymorphisms per patient were sufficiently informative to establish whether a deletion or UPD was present, the parent of origin, the size of the deletion, or if the patient was non-deleted, non-disomic. In one case the proband (an unbalanced translocation carrier with a cytogenetic deletion) had died and skin fibroblasts were tested with fluorescence in situ hybridisation (FISH) using probe GABRB3.

DEFINITIONS

Epilepsy improvement was defined as decreased frequency of seizures or reduced requirement for medication. Disruptive sleep was sleep truncated to two to three hour periods during the night with wakefulness and activity in between. Pigmentation of the skin, eye and hair colouring was subjectively assessed by two clinicians and compared with the family. Term deliveries were those between 37 and 42 weeks' gestation.

Results

GENETIC TESTING

Twenty-seven patients had deletional AS. In 26 patients with karyotype 46,XX or 46,XY, the deletion was de novo, maternal in origin, and spanning markers from D15S9 to D15S12. Not all patients were informative for each probe or had each one tested, but there was sufficient information in each case.
to establish that the deletion spanned the whole AS region and was maternal in origin. In one patient the deletion arose as the result of an unbalanced de novo translocation, 45,XY,t(10;15)(q26;q13). This patient had died aged 24 years and the cytogenetic deletion was substantiated with FISH studies on skin fibroblast cells retrieved from liquid nitrogen storage after death.

**PATIENTS**

Patient data are shown in table 1. There were 18 females and nine males. The mean age at diagnosis was 8-8 years (range 1-5 years to 32 years) and mean age at last review was 11.2 years (range 3 to 34 years). Age distribution is shown in fig 1. All patients were severely mentally retarded and had ataxic movements involving the upper and lower limbs. In patients who were walking, the ataxia manifested variously as a wide based gait, unsteadiness with jerky or clumsy movements, or a thumping heavy gait, more noticeable in the adults when excited or running. Jerky movements of the upper limbs with poor coordination were also observed. Up to six single words were reported in five children. One child (patient 20) had use of 27 single words reported by the parents but not verified by others.

**PIGMENTATION**

Hypopigmentation relative to family members was considered present in 19/26 patients (73%), while 7/26 had the same colouring as their family, including one dark skinned, black haired, brown eyed male of Italian descent. One female patient, aged 18 years, had oculo-cutaneous albinism diagnosed in infancy on the basis of characteristic retinal changes. Red hair was present in three girls, although in each case this was familial.

**PREGNANCY**

Overall there had been 90 pregnancies in the 27 families, with 13 known miscarriages (in 11 families) and 52 normal unaffected sibs. The mother's age at conception of the AS proband ranged from 23 to 36 years (mean 28, median 25 years) and the father's age at conception ranged from 25 to 44 years (mean 31, median 30 years). Of the 27 AS pregnancies, 22 continued to term, three were preterm (32, 34, and 36 weeks gestation by dates), and in two the gestation was unknown.

**ANTHROPOMETRICS AT BIRTH**

Birth weight was known in 24 patients (fig 2). For the 21 term infants the mean birth weight was 3.1 kg (range 2.5 to 3.8 kg) which fell within the normal 2nd to 98th centile range. However, there was clustering below the 50th centile and 2/21 (9.5%) had a birth weight below the 10th centile. The preterm infant born at 32 weeks (by dates) weighed 2500 g, which was above the 90th centile. Head circumferences (HC) at birth were measured in 16 patients and in two others were recorded as "normal". These 16 HC were within normal centiles (2nd to 98th) with mean HC 34.2 cm (range 33–36.8 cm). Thus, no child was microcephalic at birth. However, 11/16 (69%) were below the 50th centile, a distribution of birth HC significantly skewed from the normal (p<0.01) (fig 3).

There were insufficient recorded data on birth lengths for meaningful analysis.

**GROWTH**

Height at last review showed that 15/26 were on the 3rd centile or less (58%), six were between the 3rd and 50th centile (23%), and 5/26 were on the 50th centile or over (19%). Although 42% were not short, height distribution was significantly skewed from the normal (p<0.01). Four patients were obese,
three males and one female. One male (patient 12) weighed 150% of expected weight for height at 7 years, one male (patient 15) weighed 200% of expected weight for height at 24 years, one male (patient 8) weighed 113% of expected weight for height at 7 years of age, and one female (patient 7) weighed 180% of expected weight for height at 12-5 years. The remainder showed appropriate weight gain for height. The HC in 20/24 patients was small (less than the 25th centile), in one it was between the 25th and 50th centile, and 3/24 patients (12-5%) had a HC >50th centile (patients 7, 9, and 15) at ages 11, 18, and 24 years. Of 15 patients whose serial HC measurements were known, eight (53%) were microcephalic by 2 years of age and seven (46%) showed downward crossing of centiles approaching the 2nd centile at last review.

NEUROBEHAVIOUR
Neurological abnormalities were non-specific in infancy. Hypotonia at birth (floppy baby) was reported in 15/24 (63%), one infant was hypertonic (4%), and eight (33%) had normal tone. Feeding difficulties were reported in 20/26 (77%) babies and included difficulty with sucking, breast attachment, tongue thrust, vomiting, and reflux. Reflux in one patient (12) was managed by fundoplication at 6 months of age. The mothers of two female babies considered them “perfect”.

Gross motor skills were delayed. No child walked before 18 months (fig 4). Overall, 22 patients could walk independently and five were non-walkers. The ages of the five who were not walking independently were 3, 5, 6, 12, and 24 years. Cumulatively, 10/27 children (37%) were walking independently in their third year, 14 (52%) in the fourth year, 19 (70%) in the fifth year, and 22 (81%) by 10 years of age.

Epilepsy developed in 26/27 patients (96%). The child without overt epilepsy had an abnormal EEG and at the age of 3 years had night screaming and startles which responded to Frisium (clobazam). The age of onset of epilepsy was precisely known in 24 cases and epilepsy had developed by 3 years of age in 20 (83%). One girl had her first seizure at 8 years of age, presenting in status epilepticus lasting 1-5 hours. From the available histories, an early childhood onset of epilepsy seems likely in the remaining two patients; in one (patient 23) epilepsy was well established at 9 years, and the other, an institutionalised patient (patient 22), had “always” had epilepsy.

Epilepsy improvement was reported in 11/16 (69%) patients; in eight there were insufficient data to assess this parameter, in two the onset was recent, and one did not have epilepsy. Of the 11 patients, improvement by the age of 5 years was evident in two, by 10 years in a further five, by 15 years in a further three patients, and one patient improved as an adult. The onset of epilepsy was not associated with regression of any developmental skills attained.

An EEG had been performed in 25 patients, of which all were recorded as abnormal. One patient had an abnormal EEG but no overt epilepsy. It was not possible to draw conclusions about the type of EEG abnormality seen, owing to variations in reporting between centres and within centres over the time period of the study, or to draw any conclusions about improvement in the EEG patterns, although a number of patients had had repeat EEG studies performed.

Overall the patients slept poorly. Information on sleeping patterns was available for 21/27 patients. There were no sleeping problems in three, but the remaining 18 had disruptive sleep. A spontaneous improvement in sleep patterns was reported in 9/12 of these patients around the age of 6 to 8 years although there was no sleep improvement in two 18 year olds.

Assessment of Activities of Daily Living showed that the teenage children and the five adults were all dependent, requiring assistance with feeding, toileting, and dressing.

Discussion
From the referrals for DNA testing in patients with AS around Australasia2031 we have followed up and present here the clinical findings in 27 consecutive patients, all of whom have DNA deletions spanning known genetic loci within the PWS/AS region.222432 The clinical features of AS patients with proven DNA deletions have previously been documented in one study of 37 Japanese patients.27 This study (which includes two25 and 1126 deletional cases previously reported) is the only one providing data comparable to ours. Comparisons can be made for those features documented by both groups. Other features reported here extend the deletional phenotype in AS.

Similarities are apparent in many of the features from our data and those from Japan27 in both occurrence and frequency. The mean age of the Japanese patients (8-6 years) was slightly younger than ours (11-2 years at last review). Both groups showed severe mental retardation, ataxia, epilepsy, abnormal EEG, absent language, paroxysmal laughter, normal birth weight and head circumference, normal de-
Clinical features in 27 patients with Angelman syndrome resulting from DNA deletion

Apart from the diagnostic and prognostic aspects, the importance of presenting the clinical profile of deletional AS lies in the provision of data which can be used as a benchmark against which cases with other genetic mechanisms can be assessed. No differences were found in the Japanese data between the deletional and non-deletional cases.27 The suggestion that the phenotype of AS resulting from UPD is milder than in deletional AS has no parameters for comparison.38 In the two cases of UPD reported,37 aged 7·5 and 10·25 years, both were of small height and HC, had seizures and an abnormal EEG, but the ataxia was mild. Other cases have been reported with ataxia, but no seizures at ages 3·3, and 4·5 years and one adult had epilepsy.16 It is of interest that, currently, few data are available comparing deletional PWS with PWS resulting from UPD but the suggestion is that there are no significant differences. Detailed clinical descriptions of further cases of non-deletional AS need to be documented and the features compared with deletional AS patients, to provide validity to any conclusions drawn.

We thank Dr C. Burke, Dr A. Bye, Dr M. Edwards, Dr R. Leitner, Professor G. Morgan, Dr J. Nelson, Dr M. Parington, Dr A. Turner, Professor G. Turner, Dr E. Wilkinson, and Dr G. Wise for referring patients and help with follow up. Professor D. Sillence, Head, Department of Genetics, Children’s Hospital, for his support; Reckitt and Colman Pharmaceuticals, supporting Angelman Syndrome Research. The DNA work was performed under an NHMRC Grant.


liveries at term, and characteristic facial features in virtually 100% of patients. A similar frequency of hypopigmentation was also found. The major difference between the two studies was in the anthropometric measurements of HC and height. While the measurements made are expressed differently making direct comparison difficult, in our study there was a small HC in 83% compared with 10/29 (34·5%) in the Japanese group with microcephaly, and conversely 42·% of our patients were not short in stature compared with a height of -1·1 SD in 32/37 (86·5%) Japanese patients. There is also a difference in the sex ratio of patients, with equal distribution in the Japanese series, but with a male to female ratio in our group of 1:2. Other studies have reported an excess of females.57,10 It may be that the syndrome is more readily recognised in females than males. Sex differences could affect growth measurements and further studies of deletional patients would add data on whether there is a growth difference in males and females.

In our group of patients, features not previously described in deletional cases include the frequent occurrence of disruptive sleep (85%) and the subsequent improvement in sleep patterns with age. Sleep problems were common in the whole UK group,10 but have not been discussed in other surveys. Also noteworthy in our deletional cases was the hypotonia at birth, early smiling in infancy, and happy disposition later. The hypotonia at birth and the subsequent finding of a cytogenetic deletion could lead to a provisional diagnosis of Prader–Willi syndrome (PWS),15 so accurate early diagnosis may require DNA testing for parent of origin of the deletion.36,37 Our patients had an early age of onset of epilepsy and a late age of walking. These latter features are well known in AS as a whole.10 Improvement in epilepsy in our group of patients is an important finding, as it may indicate a degree of brain maturation, a concept supported by the improvement in the EEG patterns found in 7/7 cytogenetically deleted patients studied in Japan.9 The predictions which can be made about the improvement in epilepsy and sleep patterns may help some families when a deletion is found.

The data presented here on 27 patients suggest that there is a distinct phenotype associated with deletional AS. As a group these patients have similar features to those reported in other reviews but with a higher frequency of the same features. The focused phenotype consists of severe intellectual disability, ataxia, lack of speech, happy disposition, and epilepsy in virtually 100% of patients. There is lack of a specific neonatal phenotype, with normal birth weight and head circumference, but hypotonia and feeding difficulties are common. With time there is development of the large mouth/chin, delayed motor milestones, postnatal growth failure, abnormal sleep patterns, paroxysmal laughter, and an abnormal EEG. However, there was still some phenotypic variability in certain features, such as HC, weight, and height and the diagnosis should not be discarded if these features are not typical.
28 Smith A, Prasad M, Deng ZM, Robson L, Woodage T, Trent RJ. Comparison of high resolution cytogenetics, fluorescence in situ hybridisation (FISH) and DNA studies to validate the diagnosis of Prader-Willi and Angelman syndrome. Arch Dis Child 1995;72:397-401.