

Duplication of chromosome 15 in the region 15q11-13 in a patient with developmental delay and ataxia with similarities to Angelman syndrome

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Abstract

Duplications of the proximal long arm of chromosome 15 have been seen in the Prader-Willi syndrome (PWS), and in subjects without the Prader-Willi phenotype but with other clinical features including short stature, diabetes, anal and jejunal atresia, and acanthosis nigricans. The non-PWS subjects all had different phenotypes despite the identical findings on cytogenetic analysis. A normal phenotype has also been observed in patients with similar duplications. We report a further patient with a duplication of 15q11-13 which was detected cytogenetically and confirmed on molecular genetic analysis. She has developmental delay, particularly concerning the acquisition of speech, and an ataxic gait. These are interesting clinical features in view of the association of Angelman syndrome with abnormalities of 15q11-13.

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Deletions, duplications, and other chromosomal rearrangements of chromosome 15q11.2 have been reported frequently in the Prader-Willi syndrome.^{1,2} Cytogenetic deletions, inversions, and translocations involving this area of proximal 15q have also been reported in subjects with Angelman syndrome (AS).³⁻⁶ To date, Angelman syndrome has not been found in association with a duplication of 15q11-13. Cytogenetic examination of this region may be difficult as there is considerable normal variation in appearances, particularly of the pale band 15q11.2.⁵ A long 15q11.2 band may therefore occasionally be interpreted as abnormal if this is not taken into account. Comparison of the appearance with the chromosomes 15 of the parents may safeguard against this error. We report a patient who has a de novo duplication of the 15q11-13 region, initially detected on cytogenetic examination and confirmed by DNA analysis, and shown to involve the locus *D15S10* which is associated with the critical region in AS. It is of interest that she has significant developmental delay, particularly in the area of language, and was noted from 2 years of age to have a wide based, ataxic gait. These clinical features are seen to a more severe degree in Angelman syndrome.⁷

Case report

The proband is the first born child of unrelated, healthy parents and weighed 3630 g at term. She has a normal younger sister. The pregnancy and neonatal period were uneventful and she smiled at 12 weeks of age, sat unsupported at 9 months, and walked independently when she was 19 months old.

At 2 years of age she came to medical attention because of delay in language development with poor vocalisation and no recognisable words. On examination she was a tall, muscular girl with an ataxic 'bobbing' gait and a tendency to fall frequently. She was lax jointed and had difficulty with gross motor coordination. When reviewed at the age of 4 years (fig 1) she continued to have a wide based, ataxic gait. Her height and weight were on the 87th centile and her OFC on the 50th centile. She had two small café au lait patches on her skin but did not appear dysmorphic. She was now able to say some single words. An EEG and CT scan were both normal. She had ginger hair and blue eyes but was not significantly hypopigmented compared to the other family members. At 6 years of age she is globally developmentally delayed, functioning at a 3 year level.

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Figure 1 The proband aged 4½ years.

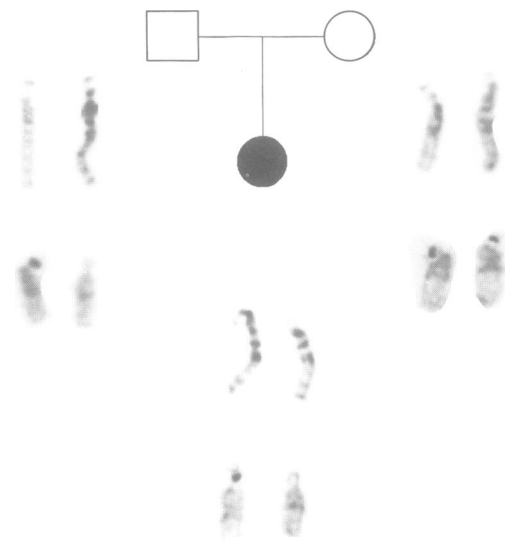


Figure 2 Partial karyotype showing chromosome 15 in the family. The proband has a large amount of extra material in proximal 15q. The 'inserted' chromosome is on the left and an extra band is shown both by GTG banding (upper) and in the Ag|NOR stained chromosome (lower). The normal chromosome 15 (right) does not stain with silver. The chromosomes 15 are uninformative as to short arms and satellites as they are similar in size and staining. Both parents have chromosomes 15 with similar sized short arms and all are satellited. None of them shows extra material in proximal 15q. However, both the maternal chromosomes 15 have Ag|NOR staining while only one of the paternal chromosomes has. Thus the normal, non-silver staining chromosome 15 in the affected daughter has been inherited from her father, and the 15 with extra material was therefore inherited from her mother.

She is now able to say some simple sentences and is a happy child with no behavioural problems.

Cytogenetic studies

High resolution cytogenetic analyses were performed on the proband and on both of her parents. This showed a de novo duplication of chromosome 15 extending from 15q11 to q13 in the proband (fig 2). Parental chromosomes were normal and based on the pattern of silver staining of the nucleolus organiser regions (Ag|NOR) the duplication is most likely on the chromosome 15 inherited from the mother.

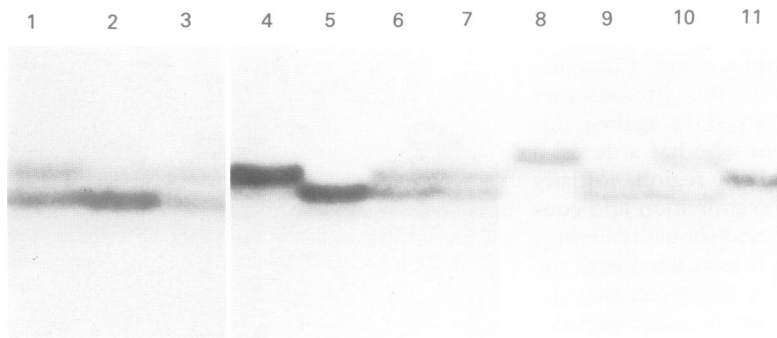


Figure 3 *D15S10* (TD3.21) alleles: track 1 mother; track 2 proband; tracks 3, 6, 7, 9 controls heterozygous for the 181 and 179 bp alleles; tracks 4, 5, 11, homozygous controls; tracks 8 and 10, examples of the 183 bp alleles. In track 2 the greatly increased intensity of the lower band can be observed. The heterozygous controls show similar intensities of the two bands.

Molecular genetic studies

METHODS

The CA repeat at the *D15S10* (pTD3-21) locus on chromosome 15⁸ was analysed in the proband, both parents, and controls using the polymerase chain reaction technique. The reported polymorphism consists of two amplified bands of 179 and 181 base pairs. We have also observed a third allele of 183 bp at a frequency of 2% and a fourth allele of 161 bp once in 256 chromosomes. DNA amplification was carried out in a 50 μ l volume containing 25 to 100 ng of genomic DNA template extracted from peripheral blood lymphocytes.⁹ The reaction solution contained 25 pmol of each oligodeoxynucleotide primer, 200 μ mol/l dATP, TTP, and dGTP, 20 μ mol/l dCTP (Pharmacia UK), 1 μ Ci α^{32} P-dCTP (3000 Ci/mmol), 50 mmol/l KCl, 10 mmol/l Tris (pH 8.8), 1.5 mmol/l MgCl₂, 0.1% Triton X-100, and 0.5 to 1.0 units of *Taq* Polymerase (Promega). Samples were overlaid with mineral oil and after initial denaturations at 94°C for 10 minutes were processed through 30 temperature cycles at 94°C (30 seconds), 56°C (20 seconds), and 72°C (30 seconds) in a PHC-2 thermocycler (Techne). The reaction mix was diluted 5 to 10 fold and 2 μ l mixed with 2 μ l sequencing stop solution. The PCR products were run on a 6% sequencing gel for two hours at 55 W.

RESULTS

Fig 3 shows an autoradiograph of amplified DNA from the proband, her mother, and several controls. The father was homozygous lower and, therefore, uninformative. Although a slight shadow is seen below each band, probably from the GT strand, comparison of the proband's track with controls shows that no other subject shows this increased density of signal in a heterozygote, indicating increased copy number of sequences in the *D15S10* region.

Discussion

The de novo duplication of 15q11-13 was detected by both cytogenetic and molecular genetic techniques, and as extensive tests have failed to show another cause for the proband's developmental delay, it seems reasonable to assume that this abnormality is the cause of her developmental problems and ataxia. Shohat *et al*¹⁰ described a case with an obesity syndrome who also had a de novo duplication of 15q11-13 and reviewed 10 other patients. Rauch and Nevin¹¹ reported a further five patients. The clinical features of all 16 patients are documented in the table along with those of our patient. None was phenotypically similar to our patient. Rauch and Nevin¹¹ also commented that they had seen similar cytogenetic abnormalities in two normal subjects. It is possible that in some of these patients the apparent extra material on chromosome 15 was in fact because of normal variation in the length of 15q11.2 without necessarily having any material duplicated. The clinical problems

Previously reported cases of 15q11-13 duplication.

Author	Age	Phenotype	Duplication
De France <i>et al</i> ¹²	6 y	Typical PWS	q12-q15
Fuhrmann-Rieger <i>et al</i> ¹³	12 y	Short stature, diabetes, large incisors, ? Cohen	q11-q13
Stallard and Van Dyke ¹⁴	Fetus	Apparently normal	q11.2-q13.3
Stallard and Van Dyke ¹⁴	Stillborn	Anal atresia, renal and skeletal abnormalities	q11.2-q13
Pattigrew <i>et al</i> ¹⁵	9 y	Typical PWS	?q11.2-q13 or q12-q13
Pattigrew <i>et al</i> ¹⁵	13 y	Cleft palate, mild mental retardation	q11.2-q13/q12-q13
Brookwell and Veleba ¹⁶	10 y	Short stature, clinodactyly, mild mental retardation	q11-q13
Brookwell and Veleba ¹⁶	4 mth	Jejunal atresia, brain malformations	q11-q13
Brookwell and Veleba ¹⁶	34 y	Normal phenotype	q11-q13
Barry <i>et al</i> ¹⁷	13 y	Typical PWS	q11.2-q13.3
Shohat <i>et al</i> ¹⁰	26 y	Obesity, hypothyroidism, acanthosis nigricans, mental retardation	q11.2-q13
Rauch and Nevin ¹¹	Neonate	Encephalocoele, cystic brain lesion	q11.2-q13
Rauch and Nevin ¹¹	?	Similar to PWS	q11.2-q13
Rauch and Nevin ¹¹	?	Primary amenorrhoea	q11.2-q13
Rauch and Nevin ¹¹	?	Insulin dependent diabetes, epilepsy	q11.2-q13
Rauch and Nevin ¹¹	?	Personality disorder	q11.2-q13
Rauch and Nevin ¹¹	?	Normal phenotype, infertility	q11.2-q13
Clayton-Smith <i>et al</i> (this paper)	6 y	Ataxia, mild mental retardation, speech delay	q11.2-q13

would therefore have another cause. Alternatively the extra material may not have been derived from chromosome 15. This would be difficult to prove without molecular genetic studies. Most deletion cases of AS (and PWS) involve large deletions including DNA probes *D15S9*, *D15S10*, *D15S11*, *D15S12*, and *D15S13*.¹⁸ However, a report of three cases within one family found a much smaller deletion involving *D15S10*¹⁹ inherited through the grandfather to the mother without phenotypic effect, and passed on by the mother to three affected children. *D15S10* may, therefore, be regarded as close to a 'critical' region in AS. In this case we have shown that *D15S10* is duplicated.

It is now well established that Prader-Willi syndrome can result from a deletion of the paternally derived chromosome 15 whereas in Angelman syndrome the deletion is maternally derived.²⁰ A reasonable hypothesis, therefore, is that the parental origin of a chromosome 15q11-q13 duplication influences the resulting phenotype. Berry *et al*¹⁷ reported a patient with classical PWS who inherited a 15q duplication from his normal father. Given that our patient has some clinical features in common with Angelman syndrome we might therefore expect that the duplication would be of the other parental origin, that is, maternal. The

cytogenetic studies suggest this is the case. Molecular studies do not so far provide any information on the parental origin of the proband's duplication but prove that the extra material is derived from 15q. A lymphoblastoid cell line is available from the proband.

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