

ELECTRONIC LETTER

Exceptionally mild Angelman syndrome phenotype associated with an incomplete imprinting defect

K Brockmann, R Böhm, J Bürger

J Med Genet 2002;**39**:e51 (<http://www.jmedgenet.com/cgi/content/full/39/9/e51>)

Angelman syndrome (AS) is a relatively frequent disorder of mental and motor development. Affected subjects show severe mental retardation, delayed motor development, movement or balance disorders with ataxic gait and jerky limb movements, and absence of speech. In addition, distinct behavioural features, such as frequent laughter and hyperactivity, microcephaly, seizures, and EEG abnormalities are typically found.¹

AS is caused by the loss of function of the maternal *UBE3A* gene. Structural mutations of the *UBE3A* gene are found in AS patients, suggesting that *UBE3A* is the major AS gene.^{2,3} More than two thirds of AS patients have a de novo deletion of approximately 4 Mb of the maternal chromosome region 15q11-q13, which affects several imprinted genes including *UBE3A* and *SNRPN*. Only about 1% of AS cases are the result of paternal disomy of chromosome 15. Finally, approximately 5% of AS patients have an imprinting defect (ID). Apparently normal chromosomes of biparental origin carry uniparental DNA methylation because of a maternal chromosome that erroneously carries a paternal methylation pattern.⁴ In some of these patients the incorrect epigenotype is caused by a deletion in the imprinting centre,^{5,6} but other mechanisms must exist as well.⁷

Several investigations with small numbers of AS patients suggested a genotype-phenotype correlation. Deletions appeared to correlate with a more severe phenotype than the other three mutation types. AS patients with imprinting defects showed microcephaly and hypopigmentation less frequently,⁸ those with *UBE3A* mutations were less severe affected than deletion patients,⁹ and AS patients with uniparental disomy showed better verbal development compared to deletion patients.¹⁰ Furthermore, an intermediate phenotype that more resembles Prader-Willi syndrome than AS was shown to be associated with AS imprinting defects.¹¹

Recently, Lossie *et al*¹² reported a distinct genotype-phenotype correlation resulting from analysis of a large series of AS patients. Most severely affected were those children with deletions of 15q11-q13, while paternal uniparental disomy of chromosome 15 and imprinting defects were associated with the least severe phenotypes.

Here, we report the association of an exceptionally mild AS phenotype with an incomplete loss of maternal methylation.

CASE REPORT

A girl, 27 months old, presented for investigation of delay in speech development. She was the third child of unrelated, healthy parents. She had no family history of note and her older sister and brother had normal speech development. After an uneventful pregnancy she was born at term with a weight of 3570 g and head circumference of 35 cm. She was crawling at 8 months and walking at 14 months. As an infant, she vocalised little and produced few double syllables. At 18 months she said "mama" and "papa" occasionally, but gained no other words over one year. Her understanding was much better and she fetched things on request. She could chew and

swallow normally, showing mild drooling. She exhibited an exceptionally happy demeanour and frequent laughing, but no inappropriate bursts of laughter. She would play on her own and showed simple role playing.

On examination at 27 months, her gait was slightly stiff, toddling, and cautious, with her arms in a flexed position. She could not jump on both legs, but was able to avoid obstacles. She was able to build a block tower, turn over single pages, unwrap a sweet, but did not have pincer grasp. She picked up toys from the ground in unsupported squat. Her muscle tone and tendon reflexes were normal. She had blonde hair and blue eyes. Her tongue was not protruding and there was no prognathism or strabismus. Body asymmetry and skin pigmentary anomalies which might suggest mosaicism were not found.

Her weight and height were on the 97th centile and her body mass index (BMI) was 18 (90th centile). Her head circumference was on the 25th centile.

On follow up at the age of 3 years, she showed more secure and fluid movements. She could ride a tricycle and scooter (fig 1), play soccer, and climb stairs without holding on. She could eat and drink independently, butter a slice of bread, and pour water without spilling. She spoke 20 single words and several two word sentences, otherwise communicating using gestures and sounds. Her comprehension skills appeared nearly

Key points

- Angelman syndrome (AS) is a disorder of psychomotor development caused by loss of function of the imprinted *UBE3A* gene. Since the paternal *UBE3A* copy is regularly silent, only mutations inactivating the maternal copy cause AS.
- We report a 3 year old girl with unusually mild clinical symptoms of AS, exceptional speech skills, and motor performance almost adequate for age. EEG showed rhythmic high amplitude slow waves facilitated by eye closure.
- Initial DNA methylation testing at the *SNRPN* locus showed an incomplete loss of maternal methylation. Microsatellites showed normal biparental inheritance. A mutation of the imprinting centre was not found. Thus the patient is mosaic for a sporadic imprinting defect on the maternal chromosome.
- Approximately 10% of leucocytes appear to carry a normal methylation pattern. Whether neurones are also mosaic in the patient is not known. If so, 10% active *UBE3A* could explain the very mild phenotype.
- Our observations further widen the clinical spectrum of AS. To enable optimal therapy and exact genetic counselling, molecular diagnosis for AS should be performed even when clinical signs are mild or atypical.



Figure 1 Girl with Angelman syndrome at 3 years 10 months of age.

adequate for her age. No seizures had been observed. Her BMI was now 17 (75th centile).

Electroencephalography performed at 30 months showed runs of high amplitude (200-400 μ V) rhythmic 2-4/s activity more prominent occipitally and provoked by eye closure. No spikes were observed.

Speech delay, mild ataxia, and exceptionally happy demeanour in association with these characteristic EEG abnormalities drew the clinician's attention to the possibility of AS.

DNA of the patient analysed for methylation at *SNRPN*¹³ showed a methylation pattern with a normal paternal and a weak maternal band. We estimate the maternal band to be 10% of the paternal one. Microsatellite analysis⁷ showed a normal biparental inheritance of parental alleles (D15S128: father 8, mother 6, patient 6-8; D15S986: father 5-10, mother 8-10, patient 5-8; D15S1234: father 7-12, mother 7-11, patient 7). Quantitative Southern blot analysis for the shortest region of deletion overlap of imprinting centre deletions, performed as described elsewhere,¹⁴ showed a normal dose in the patient.

The patient had a normal biparental inheritance at the AS/PWS locus and a normal gene dosage at the imprinting centre. These results exclude three mutation types that cause AS: (1) deletion of 15q11-q13, (2) uniparental disomy, and (3) imprinting mutations. On the other hand, the maternal methylation signal of this patient was reduced to approximately 10%. These findings were suggestive of an incomplete imprinting defect.

DISCUSSION

In normal subjects, the maternal *SNRPN* allele is methylated and the paternal allele is unmethylated. Partial hypomethylation of the *SNRPN* locus in the patient indicates that she is mosaic for a sporadic imprinting defect on the maternal chromosome.

Within a cell, the *SNRPN* locus is either methylated or not. Therefore, a quantitative ratio of 90 to 10 can only be obtained by a mix of two different cell populations; 10% residual methylation means that 10% of the analysed cells are methylated at *SNRPN* and 90% are not. Hence, the patient carries an epigenetic mosaic in her leucocytes.

The lack of an IC deletion and the presence of a mosaic methylation pattern in the patient point to a postzygotic

defect. This is compatible with a report by El-Maarri *et al*,¹⁵ who showed that the maternal methylation imprint on human chromosome 15 is established only at or after fertilisation.

Our findings raise the question whether the mild phenotype of the patient is a coincidence or causally related to the incomplete imprinting defect. As the AS phenotype does not depend on methylation and *UBE3A* gene expression in blood leucocytes, we have no answer to this question. Vu *et al*¹⁶ showed predominant expression from one *UBE3A* allele in brain and Rougeulle *et al*¹⁷ showed that *UBE3A* expression in AS frontal cortex is reduced. Murine *Ube3a* imprinting is restricted to distinct regions of the brain.¹⁸ Reduction of *UBE3A* gene expression in exactly these brain regions seems to determine the AS phenotype. Whether these neurones are also mosaic in our patient is not known and does not necessarily follow from our findings in leucocytes. However, it may be assumed that 10% active *UBE3A* would result in a milder phenotype than no active *UBE3A* at all. Whether the imprinting status in neurones correlates with the severity of the phenotype can be answered only by methylation testing of neurones of AS patients with ID.

Gillessen-Kaesbach *et al*¹¹ reported seven patients with a previously unrecognised phenotype of Angelman syndrome caused by an imprinting defect. Clinical features of these children comprised obesity, muscular hypotonia, mild mental retardation, and ability to speak, thus resembling the phenotype of Prader-Willi syndrome. EEG changes characteristic of AS were observed in five patients. Our patient shares in common with these children the mild mental retardation and the EEG abnormalities, but she did not show muscular hypotonia or obesity. Five of these seven children had body mass indices (BMI) (as calculated from body weight and height data given in Gillessen-Kaesbach *et al*¹¹) above the 97th centile, while the BMI of two children was on the 90th centile, as it was in our patient.

The clinical concept of AS as a disorder that consistently exhibits severe developmental delay, distinct speech impairment with no or minimal use of words, and ataxia needs to be modified.^{11, 19, 20} The vast majority of AS patients will still meet the classical clinical criteria. However, with increasing awareness of AS by physicians, a growing number of children are detected with atypical features. Our observation further widens the clinical spectrum of AS. In our patient, the clinical features of AS are unusually mild and the main diagnostic criteria are lacking. She reached milestones of motor development within normal limits. A mild ataxia, present at the beginning of her third year, had nearly completely resolved at follow up a year later. Her active speech skills comprising 20 words at the age of 3 years are exceptional in children with AS. We would like to stress the importance of recording an EEG with active or passive eye closure in every child with mental retardation.

Based on this observation we suggest performing molecular genetic investigation for AS even if clinical signs are mild or atypical. The identification of AS patients with atypical phenotypes will help to widen our understanding of the mysterious mechanisms of imprinting defects. Furthermore, only a confirmed diagnosis enables better patient management and exact genetic counselling of the parents.

Authors' affiliations

K Brockmann, R Böhm*, Department of Paediatrics and Neuropaediatrics, Children's Hospital, Georg-August-University, Göttingen, Germany

J Bürgert†, Institute of Human Genetics, Virchow-Klinikum, Humboldt University, Berlin, Germany

Correspondence to: Dr K Brockmann, Department of Paediatrics and Neuropaediatrics, Children's Hospital, Robert-Koch-Strasse 40, D-37075

Göttingen, Germany; kbrock@med.uni-goettingen.de

*Present address: Children's Hospital Gilead, Bielefeld-Bethel, Germany

†Present address: Munich Re, Life Sciences Centre of Competence, München, Germany

REFERENCES

- 1 **Williams CA**, Angelman H, Clayton-Smith J, Driscoll DJ, Hendrickson JE, Knoll JH, Magenis RE, Schinzel A, Wagstaff J, Whidden EM, Zori R. Angelman syndrome: consensus for diagnostic criteria. *Am J Med Genet* 1995;**56**:237-38.
- 2 **Kishino T**, Lalonde M, Wagstaff J. UBE3A/E6-AP mutations cause Angelman syndrome. *Nat Genet* 1997;**15**:70-3.
- 3 **Matsuura T**, Sutcliffe JS, Fang P, Galjaard RJ, Jiang YH, Benton CS, Rommens JM, Beaudet AL. De novo truncating mutations in E6-AP ubiquitin-protein ligase gene (UBE3A) in Angelman syndrome. *Nat Genet* 1997;**15**:74-7.
- 4 **Reis A**, Dittrich B, Greger V, Buiting K, Lalonde M, Gillissen-Kaesbach G, Anvret M, Horsthemke B. Imprinting mutations suggested by abnormal DNA methylation patterns in familial Angelman and Prader-Willi syndromes. *Am J Hum Genet* 1994;**54**:741-7.
- 5 **Sutcliffe JS**, Nakao M, Christian S, Orstavik KH, Tommerup N, Ledbetter DH, Beaudet AL. Deletions of a differentially methylated CpG island at the SNRPN gene define a putative imprinting control region. *Nat Genet* 1994;**8**:52-8.
- 6 **Buiting K**, Saitoh S, Gross S, Dittrich B, Schwartz S, Nicholls RD, Horsthemke B. Inherited microdeletions in the Angelman and Prader-Willi syndromes define an imprinting centre on human chromosome 15. *Nat Genet* 1995;**9**:395-400.
- 7 **Bürger J**, Buiting K, Dittrich B, Gross S, Lich C, Sperling K, Reis A, Horsthemke B. Different mechanisms and recurrence risks of imprinting defects in Angelman syndrome. *Am J Hum Genet* 1997;**61**:88-93.
- 8 **Bürger J**, Kunze J, Sperling K, Reis A. Phenotypic differences in Angelman syndrome patients: imprinting mutations show less frequently microcephaly and hypopigmentation than deletions. *Am J Med Genet* 1996;**66**:221-6.
- 9 **Moncla A**, Malzac P, Voelckel MA, Auquier P, Girardot L, Mattei MG, Philip N, Mattei JF, Lalonde M, Livet MO. Phenotype-genotype correlation in 20 deletion and 20 non-deletion Angelman syndrome patients. *Eur J Hum Genet* 1999;**7**:131-9.
- 10 **Fridman C**, Varela MC, Kok F, Diamant A, Koiffman CP. Paternal UPD15: further genetic and clinical studies in four Angelman syndrome patients. *Am J Med Genet* 2000;**92**:322-7.
- 11 **Gillissen-Kaesbach G**, Demuth S, Thiele H, Theile U, Lich C, Horsthemke B. A previously unrecognised phenotype characterised by obesity, muscular hypotonia, and ability to speak in patients with Angelman syndrome caused by an imprinting defect. *Eur J Hum Genet* 1999;**7**:638-44.
- 12 **Lossie AC**, Whitney MM, Amidon D, Dong HJ, Chen P, Theriaque D, Hutson A, Nicholls RD, Zori RT, Williams CA, Driscoll DJ. Distinct phenotypes distinguish the molecular classes of Angelman syndrome. *J Med Genet* 2001;**38**:834-45.
- 13 **Zeschinig M**, Lich C, Buiting K, Doerfler W, Horsthemke B. A single-tube PCR test for the diagnosis of Angelman and Prader-Willi syndrome based on allelic methylation differences at the SNRPN locus. *Eur J Hum Genet* 1997;**5**:94-8.
- 14 **Buiting K**, Lich C, Cottrell S, Barnicoat A, Horsthemke B. A 5-kb imprinting center deletion in a family with Angelman syndrome reduces the shortest region of deletion overlap to 880 bp. *Hum Genet* 1999;**105**:665-6.
- 15 **El-Maarri O**, Buiting K, Peery EG, Kroisel PM, Balaban B, Wagner K, Urman B, Brannan CI, Walter J, Horsthemke B. Maternal methylation imprints on human chromosome 15 are established around or after fertilisation. *Nat Genet* 2001;**27**:341-4.
- 16 **Vu TH**, Hoffman AR. Imprinting of the Angelman syndrome gene, UBE3A, is restricted to brain. *Nat Genet* 1997;**17**:12-13.
- 17 **Rougeulle C**, Glatt H, Lalonde M. The Angelman syndrome candidate gene, UBE3A/E6-AP, is imprinted in brain. *Nat Genet* 1997;**17**:14-15.
- 18 **Albrecht U**, Sutcliffe JS, Cattanauch BM, Beechey CV, Armstrong D, Eichele G, Beaudet AL. Imprinted expression of the murine Angelman syndrome gene, Ube3a, in hippocampal and Purkinje neurons. *Nat Genet* 1997;**17**:75-8.
- 19 **Laan LA**, van Haeringen A, Brouwer OF. Angelman syndrome: a review of clinical and genetic aspects. *Clin Neurol Neurosurg* 1999;**101**:161-70.
- 20 **Dan B**, Boyd SG, Christiaens F, Courtens W, Van Maldergem L, Kahn A. Atypical features in Angelman syndrome due to imprinting defect of uniparental disomy of chromosome 15. *Neuropediatrics* 2000;**31**:109-10.