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CASE REPORTS

Phenotypic variability in Angelman syndrome – report of two cases

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ABSTRACT

Angelman syndrome is a genetic condition, characterized by severe mental retardation, ataxic gait, severe speech delay, dysmorphic features, abnormal behaviours, movement disorder. It is caused by a variety of genetic mechanisms which all interfere with expression of the UBE3A gene on chromosome 15q11-13. In this paper, we present two cases of Angelman syndrome, one of them with classical phenotype, and the other with a Rett syndrome – like phenotype. In both patients, the molecular cytogenetic investigation confirmed the interstitial deletion within critical region 15q11-13.

Key words: Angelman syndrome, Rett syndrome, FISH

INTRODUCTION

Angelman syndrome (AS) is a complex neurodevelopmental disorder with a difficult clinical diagnosis and a heterogeneous genetic basis.

AS is characterized by mental retardation, movement or balance disorder, characteristic abnormal behaviours, and severe limitations in speech and language. Clinical features include severe motor and intellectual retardation, learning disability; ataxic, jerky movements; seizures; absent speech; happy, sociable disposition; characteristic facial features (deep set eyes; small mid-face, pointed chin; wide, smiling mouth; tongue protrusion; relatively widespaced teeth; brachycephaly); blonde hair/ blue eyes in some patients (Angelman, 1965) (1).

The individuals with AS have characteristic behavioural features: sociable; laughter easily provoked; love of water; inquisitive; fascination with reflections; sleep disorder (2).

Seizures in AS are present in 85% of patients. The onset is often at 12-18 months and includes all seizure types, which are difficult to control. There is a characteristic EEG in most but not all patients.

In adulthood most patients are healthy. Obesity is common, also oesophageal reflux can

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be present. Lack of mobility and contractures may impair health. Other features include seizures, cortical myoclonus, keratoconus (3).

Symptoms vary from one person to another. Even with the best therapy some of these complications may arise.

Scoliosis develops in 40% adults, with a highest risk in adolescent growth spurt. It needs regular surveillance; surgery may be required.

Four genetic mechanisms affecting the imprinted region 15q11-13 are recognized to cause AS. Patients are thus divided into four classes (I to IV), based on these mechanisms. Approximately 70-75% of AS cases result from de novo deletions of maternal origin involving chromosome 15q11-q13 (class I). The deletion has a similar size in most patients, aproximatelly 4 Mb, and common breakpoints. A second class of AS patients have paternal uniparental disomy of 15q11-q13 (class II, 2-3%). In 3-5% of patients an abnormal methylation pattern of chromosome 15 is detected, signifying imprinting defects (class III). Mutations in the gene encoding the ubiquitin-protein ligase E3A gene (UBE3A) occur in 10% of AS patients (4). 75% of familial AS patients have UBE3A mutations. The patients with UBE3A mutation (point mutation or small deletion) represent class IV. All genetic mechanisms involved in AS interfere with expression of the UBE3A gene on chromosome 15q11-13. UBE3A gene encodes a ubiquitin protein ligase which is involved in protein degradation within the brain. This gene is maternally imprinted in brain, expressed in hippocampus, olfactory tracts and Purkinje cells of cerebellum. The protein product, E6AP, acts as E3 ligase in ubiquitin proteosome pathway. Also, it is a transcriptional co-activator and up-regulated in neurons where it has a role in dendritic spine development (5).

In approximately 10% of AS patients none of the above-mentioned genetic defects is identified. These patients are defined as class V.

The diagnosis of AS is confirmed by genetic studies, including: methylation analysis of 15q11-13 region, karyotype and fluorescence *in situ* hybridization (FISH) for AS/Prader-Willi syndrome critical region; UPD studies (parental DNA) if cytogenetics is normal; mutation analysis of *UBE3A* if clinical featureas are typical for AS, but cytogenetics and methylation are normal, or if a family history of AS exists. ❑

CASES PRESENTATION

CASE 1 is a 12-year-old girl, admitted in our department for neurological evaluation because of a sever progressive toraco-lombar scoliosis, which occured at the age of five. She was the first child from healthy non-consanguineous parents, with uneventfull pregnancy and delivery. In the early childhood she presented delayed milestones, failure to develop speech, onset of epileptic seizures, sleep disorder. From the age of two she began to present epileptic seizures (absences and partial seizures), treated with antiepileptic drugs (ethosuxinimide and clonazepam) with partial control of the seizures. At the moment of admittion in our clinic she presented, daily, 1-2 seizures.

Clinical evaluation revealed: dysmorphic features (deep set eyes, pointed chin, wide, smiling mouth; see Figure 1); happy, sociabile disposition; toraco-lombar scoliosis.

Neurologic examination showed: ataxic gait; absent speech; autistic features, hyperventilation episodes, severe mental retardation.

Paraclinical investigations included:
- biological tests – in normal range;
- EEG – generalised sharp-waves discharge;
- Cerebral MRI scan – normal.

CASE 2 is a 4-year-old boy, admitted in our department for psychomotor retardation. He was the fifth child from healthy non-consanguineous parents, with normal pregnancy and delivery. All the other siblings are healthy. In infancy and early childhood he presented delayed milestones, failure to develop speech.

Clinical evaluation revealed: dysmorphic features (deep set eyes, pointed chin, wide, smiling mouth, relatively wide-spaced teeth, brachycephaly; see figure 3), blonde hair, blue eyes, happy, sociabile disposition.

Neurologic examination showed gait difficulties with ataxia, severe mental and language retardation (produce some syllabic sounds).

Paraclinical investigation included:
- biological tests – in normal range;
- EEG – right temporo-parietal sharp-waves discharge;
- Cerebral CT scan – normal.

Cytogenetic and FISH analysis

Metaphase spreads were obtained from short-term blood cultures by standard methods.

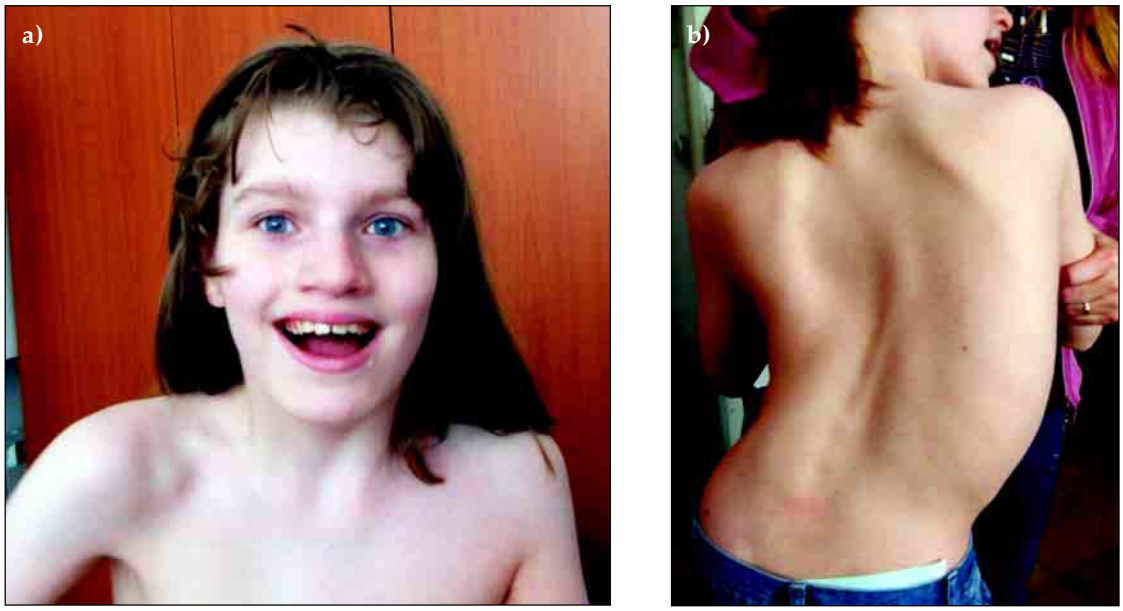

FIGURE 1. Case 1. a) Dysmorphic features: deep set eyes, pointed chin, wide, smiling mouth, blue eyes, happy, sociabile disposition; b) Severe toraco-lombar scoliosis.

FIGURE 2. FISH assay of case 1 with BAC probe RP11-1081A4 (15q11.2, green) and controle probe RP11-89K11 (15q26.3, red). The absence of green signal for RP11-1081A4 BAC probe cofirms the interstitial deletion within 15q11-13 critical region.

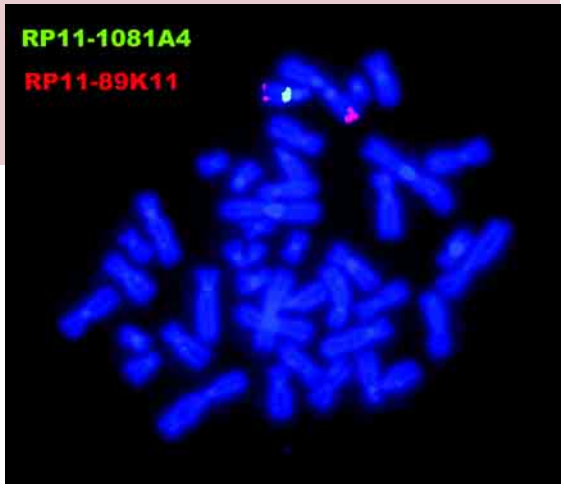

FIGURE 3. Dysmorphic features in case 2: deep set eyes, pointed chin, wide, smiling mouth, relatively wide-spaced teeth, brachycephaly), blonde hair, blue eyes, happy, sociabile disposition.

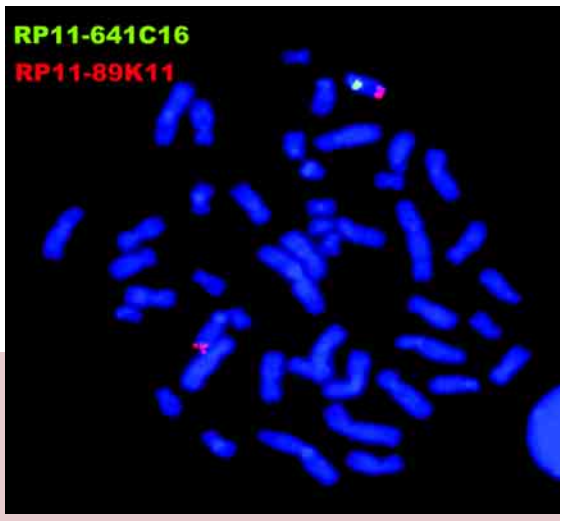

FIGURE 4. FISH assay of case 2 with BAC probe RP11-641C16 (15q11.2, green) and controle probe RP11-89K11 (15q26.3, red). The absence of green signal for RP11-641C16 BAC probe confirms the interstitial deletion within 15q11-13 critical region.

GTG banded metaphase chromosomes were analysed at 550 band resolution. For FISH studies, Bacterial Artificial Chromosomes (BACs) probes for critical region 15q11-13 and controle probes were used. Three BACs were selected from the RPCI-11 library using the NCBI Genome Browser (Map Viewer, Build 35.1) and were supplied by The Wellcome Trust Sanger Institute. BAC DNA was directly labelled with either Spectrum Green-dUTP or Rhodamine using commercially available kits (Vysis, Roche). RP11-641C16 and RP11-1081A4 BAC probes were used to investigate the critical AS region. RP11-89K11 (15q26.3 region) was selected as control probe. FISH analysis was performed on a epifluorescence microscope (NIKON E800) equiped with a CCD camera (NIKON DS-2MBWc) and a dedicated software (NIS BR Elements – LUCIA). ❑

RESULTS

Cytogenetic investigation revealed normal karyotype in both cases. FISH studies showed microdeletion within 15q11-13 critical region (figures 2 and 4), both in case 1 and case 2, which confirm the diagnosis of Angelman syndrome along with the clinical findings. In CASE 1, the molecular cytogenetic studies were performed with BAC probe RP11-1081A4 for critical region 15q11-13 and a controle probe RP11-89K11 (15q26.3). Deletion of RP11-1081A4 locus (including UBE3A gene) was detected. In CASE 2, FISH was performed with BAC probe RP11-641C16 for critical region 15q11-13 and the same controle probe RP11-89K11 (15q26.3). RP11-641C16 locus was deleted in case 2. The extension of the microdeletion in these two cases is still to be established. ❑

DISSCUSIONS

Angelman syndrome affects ~ 1/12 000 – 1/40 000 children. Diagnosis can be made by specific clinical featureas, key points of examination beeing: growth parameters including occipital-frontal circumference (OFC; most have OFC < 25th centiel by 3 years; severe microcephaly is very unusual); happy and sociable affect; wide-based, stiff-legged posture; increased muscle tone in muscle; jerky gait with arms often held with elbows bent and hands uplifted to shoulder level; excitability with characteristic hand flapping; subtile facial features including wide, smiling mouth, prominent chin, and deep-set eyes. Diagnosis confirmation is usually made by methylation analysis of the 15q11-13 region which shows absence of maternally imprinted allele. Molecular cytogenetic analysis – karyotype and FISH for AS/Prader-Willi syndrome critical region give information on the mechanism of the disease (microdeletion) which is of primary importance for genetic counselling. While FISH can be used as a confirmatory tool as well when clinical findings are clear-cut, one may keep in mind that some cases of misdiagnosis can exist when used as a standalone test and the patient presents with atypical clinical findings because it cannot recognize between deletion of paternal or maternal chromosome 15.

Maternal origin microdeletion of the imprinted region 15q11-13 is the most frequent genetic mechanism in AS. The deletion encompasses a region of 4 to 6 Mb, with a common distal breakpoint (BP3) and two proximal breakpoints (BP1 and BP2). The breakpoints, confined to a relatively small region of chromosome 15, allows for the identification of 2 classes of deletion subjects. Type I deletion involves BP1 (proximal to D15S541/S1035 loci) and BP3. Type II deletion is approximately 500 Kb smaller, and involves BP2 (between loci D15S541/S1035 and D15S543) and BP3. The distal breakpoint in the 15q11-q13 region, BP3 is located between loci D15S156 and D15S165, and is observed both in type I and in type II deletions. Recent studies revealed interesting genotype-phenotype corellations in AS patients.

The patients with larger deletion (type I) seems to have a more severe phenotype compared to patients with type II deletions. Patients with type I deletion were more likely to have the criteria for comorbid diagnosis of autism, and were significantly more likely to require more seizure medications than their class II counterpart (6,7).

Genotype/Phenotype Correlation

The Deletion Phenotype is characterised by: small head size; seizures in 95% cases; seizures more severe; greater delay/inability to walk; hypopigmentation; typical facies; more "hard" neurological signs.

The UPD Phenotype include: walk earlier (2-3 years); less ataxia; fewer seizures (20%);

better comprehension and communication; no hypopigmentation; less obvious facial features; later diagnosis; higher birth weight.

The "Imprintor" Phenotype is characterised by: fewer seizures; better communication skills; less likely to have small head size; less ataxia; no hypopigmentation; still have AS personality.

The UBE3A Phenotype include: smallish head size; walk around 3 years; seizures in 50% cases; less ataxia; no hypopigmentation; some facial features; better communication skills than deletion patients.

There are some features consistent across all groups: happy, sociable behaviour; characteristic EEG; love of water; sleep disorder (8, 9).

Differential diagnosis

A common differential diagnosis in female patients with AS is Rett syndrome (RS). Similar features of AS and RS include: ataxia; deceleration of OFC; brachycephaly; seizures and abnormal EEG; frequent laughter; stereotypic hand movements; severe LD and absent speech; scoliosis. The clinical differential diagnosis of AS and RS is based on: absence of regression, characteristic features of EEG, and specific movement disorder in AS, while RS is characterized by vasomotor instability, tremor, anxiety, absence of happy disposition. Compared with AS, RS is a progressive disorder with poorer prognosis (8).

Other differential diagnoses include: Mowat Wilson syndrome; ATR-X syndrome; X- linked disorder with mutations in XNP gene at Xq13; 22q13-qter deletion; other chromosomal abnormalities resembling AS (9q34 deletion, 5p-, 17q21 microdeletion); perisylvian syndrome; Lennox-Gastaut syndrome; ataxic cerebral palsy; ARX syndrome (8).

In the second case presented in this paper, the phenotype was highly suggestive for Angelman syndrome, while in the first case, a differential diagnosis with Rett syndrome was considered especially because of the sever vertebral scoliosis and hyperventilation episodes.

Molecular investigation of specific genetic defect allowed, in our cases, the refinement of clinical diagnosis. ❑

CONCLUSION

There are different phenotypes in Angelman syndrome, according to the genetic abnormality, but the phenotype can differ even in the same genotype. The role of epigenetic factors are, still, under disscusions. Some characteristic features, including happy and sociable behaviour, ataxic gait, severe limitations in speech and language, typical facies, should lead to clinical supposition of AS, confirmed by molecular genetic tests (FISH, PCR). An early diagnosis is very important in these patients for specific psychopedagogic intervention, prevention or early management of the complications, and genetic counselling. ❑

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REFERENCES

1. **Angelman H** – "Puppet children": a report of three cases. *Dev Med Child Neurol* 1965; 7:681-688
2. **Clayton-Smith J, Laan L** – Angelman syndrome: a review of the clinical and genetic aspects. *J Med Genet* 2003; 40 (2):87-95
3. **Williams CA** – Neurological aspects of the Angelman syndrome. *Brain Dev* 2005; 27:88-94
4. **Firth HV, Hurst JA** – Clinical Genetics. Ed Oxford University Press, New York, 2005, Chapter 3:272-273
5. **Dindot SV, Antalffy BA, Bhattacharjee MB, et al** – The Angelman syndrome ubiquitin ligase localizes to the synapse and nucleus, and maternal deficiency results in abnormal dendritic spine morphology. *Human Molecular Genetics* 2008; 17(1):111-118
6. **Butler MG, Bittel DC, Kibiryeva N, et al** – Behavioral differences among subjects with Prader-Willi syndrome and type I or type II deletion and maternal disomy. *Pediatrics* 2004; 113:565-573
7. **Sahoo T, Peters SU, Madduri NS, et al** – Microarray based comparative genomic hybridization testing in deletion bearing patients with Angelman syndrome: genotype-phenotype correlations. *J. Med. Genet*. 2006; 43(6):512-516
8. **Clayton-Smith J, Pembrey ME** – Angelman syndrome. *J Med Genet* 1992; 29:412-415
9. **Magenis RE, Brown MG, Lacy DA, et al** – Is Angelman syndrome an alternate result of del(15)(q11q13)? *Am J Med Genet* 1987; 28:829-838