

FISH ANALYSIS, ESSENTIAL TEST FOR DIAGNOSIS ELUCIDATION IN TWO CLINICALLY RESEMBLING GENETIC SYNDROMES: RETT AND ANGELMAN SYNDROMES

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REZUMAT

Prezentăm un caz în care manifestările fenotipice în acest moment al dezvoltării sunt comune sindromului Rett și sindromului Angelman. Afectarea neurologică, manifestările autiste, convulsiile și afectarea severă a vorbirii reprezintă simptomatologia clinică, nespecifică pentru una din aceste afecțiuni, la data solicitării consultului genetic. Deși au manifestări clinice asemănătoare, cele două afecțiuni au mecanisme patogenice diferite. Sindromul Rett este cauzat de mutații ale unei gene plasate pe cromozomul X, care codifică proteina MECP2, iar sindromul Angelman este determinat de o microdeleție la nivelul brațului lung al cromozomului 15. Analiza FISH utilizată pentru elucidarea diagnosticului a relevat prezența întregului material genetic pe cromozomii 15, iar analiza moleculară a confirmat prezența unei mutații R168X în heterozigoție, în exonul 3 al genei MECP2, astfel încât diagnosticul sindromului Rett s-a putut stabili fără echivoc. Diagnosticul diferențial este important nu numai pentru clinicieni, dar și pentru geneticieni, pentru sfatul genetic, cele două sindroame având modalități diferite de transmitere și riscuri de recurență diferite.

Cuvinte cheie: sindromul Rett, sindromul Angelman, diagnostic diferențial, analiza FISH, sfat genetic

ABSTRACT

We report a case with a phenotype that might occur in Rett syndrome as well as in Angelman syndrome. The neurological impairment, autism, seizures and severe speech impairment at this moment are common features of these two syndromes. Even if these conditions clinically resemble, they are due to different pathogenic mechanisms. Rett syndrome is caused by mutations in an X-linked gene coding for MECP2 protein, but Angelman syndrome is caused by a microdeletion in the long arm of chromosome 15. FISH analysis used for diagnosis elucidation, revealed the presence of all the genetic material on chromosomes 15 and molecular investigation confirmed a heterozygous mutation R168X in the exon 3 of the MECP2-gene, so the diagnosis of Rett syndrome could be precisely established. The differential diagnosis is essential not only for clinicians, but also for geneticists, for the genetic counseling, as the disorders have different inheritance patterns and different recurrence risks.

Key Words: Rett syndrome, Angelman syndrome, differential diagnosis, FISH analysis, genetic counseling

INTRODUCTION

Rett syndrome is a genetic condition that was first described by Andreas Rett in 1966. It is a neurodevelopmental disorder, but it also affects many other systems. Afflicted children often exhibit autistic-like behavior, such as repetitive hand movements, prolonged toe walking, body rocking, and sleep problems.

The incidence of Rett syndrome shows wide variations, being estimated between 1 in 10,000 and 1 in 15,000 births.

The vast majority of cases are sporadic. However, familial cases were also described, though they comprise only about 1% of the total reported cases. Females are almost exclusively affected, the best explanation being offered by the X-linked dominant inheritance with lethality in hemizygous males.¹

The syndrome is usually caused by mutations of a gene known as MECP2, which is thought to be critical in brain development.² The gene is located on the long arm of chromosome X (Xq28).^{3,4} It has been demonstrated that the gene is subject to X chromosome inactivation. The pattern of inactivation has been postulated to explain the phenotypic heterogeneity associated with MECP2 mutations. MECP2 is a four exons gene encoding two separate isoforms depending on the use of alternative splice variants. Classic Rett

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syndrome occurs with and without MECP2 mutations. It is estimated that only 80% of cases are due to mutations in the MECP2 gene.

Typical characteristics of the disorder are: normal development until 1/2 to 1 1/2 years; shakiness of the torso, and possibly of the limbs; unsteady gait; breathing difficulties (hyperventilation, apnea, air swallowing); seizures; teeth grinding; retarded growth and small head and severe to profound mental retardation.^{5,6}

Patients seem normal for the first 6-18 months of life, but then begin to lose the ability to speak or use their hands. This regression continues into autistic behavior, ataxia, repetitive hand movements and microcephaly.⁷ A staging system of the disorder has been developed, delineating the evolving symptoms. There are four stages of Rett syndrome. Stage I, the early onset stagnation, generally begins between 6 and 18 months of age. There may be delays in gross motor skills, the infant may begin to show less eye contact, hand-wringing and decreasing head growth may occur. Stage II, characterized by rapid developmental regression, usually begins between ages 1 and 4 years. The characteristic hand movements begin to emerge during this stage and often include wringing, washing, clapping, as well as repeatedly moving the hands to the mouth. Breathing irregularities, autistic-like symptoms may occur. Stage III, also called pseudo-stationary stage, usually begins between ages 2 and 10. Apraxia, motor problems, and seizures are prominent during this stage, but there may be improvement in behavior. Stage IV is called the late motor deterioration stage and can last for years or decades. It is characterized by muscle wasting, spasticity, scoliosis, complete wheelchair dependency.⁸

CASE REPORT

We report the case of a 19 months girl. The patient is the first and only child of healthy, unrelated parents. Family history was unremarkable. Pregnancy was uneventful. Birth was at term, with a birthweight of 3200 g, length 44 cm and head circumference 35 cm. Development seemed to be normal during the first 10 months. She was able to sit when she was 7 months, but at 1 year of age she could not stand up and developmental milestones were delayed. Her vocabulary consisted of seven words at about 1 year.

The child was evaluated at 19 months. Head circumference was 46 cm. She had seizures, difficulties in walking, unsteady gait, autistic-like behavior, with loss of social interaction and communicative skills, loss of oral language, and loss of purposeful finger

and hand use. Stereotypic hand movements were also present, consisting of hand wringing, and hand-to-mouth movements. Seizures, spasticity, jactatus capitis, bruxism, uncoordinated movements, happy face, mild enophthalmia, blue eyes, micrognathia are other clinical features noticed in this patient. EEG in sleep reveals theta delta activity, displaying bursts of repeated synchronous spikes and polyspikes, maximal over the frontal regions.

The diagnosis was initially made clinically on the basis of internationally accepted criteria.⁹ Mandatory criteria fulfilled by our patient were:

- Apparently normal prenatal and perinatal period;
- Apparently normal development through at least the first 5-6 mo of life;
- Normal head circumference at birth;
- Deceleration of head growth;
- Loss of acquired skills, including learned purposeful hand skills and communicative abilities;
- Hand stereotypies;
- Gait abnormalities.

Supportive criteria:

- Epilepsy;
- Spastic signs;
- Bruxism.

Exclusion criteria:

- Confirmed inborn error of metabolism;
- Confirmed neurodegenerative disorder;
- Optic atrophy;
- History of perinatal brain damage;
- Severe head trauma or infection leading to acquired neurological disorder.

MRI revealed a normal aspect.

Cytogenetic analysis

Chromosome studies were performed from peripheral blood lymphocyte cultures. Analysis of 50 GTG-banded metaphases showed the karyotype 46,XX, but at this level of resolution a small microdeletion 15q11-q13 could not be excluded. (Fig. 1) FISH studies are usually performed in conjunction with conventional cytogenetic analysis, in order to diagnose microdeletion syndromes. FISH analysis using VBE3A probes, specific for 15q11-q13 fragment, revealed that signals were present in both chromosomes 15. (Fig. 2) Thus, microdeletions were excluded.

Molecular analysis

Molecular investigation performed in Heidelberg (Gemeinschaftspraxis - Labor Dr. Limbach & Kollegen) confirmed the diagnosis: heterozygosity for the mutation R168X in the MECP2-gene, exclusion of

an Angelman syndrome. Analysis of genomic DNA from lymphocytes was performed. The exons 1 to 3 of the MECP2-gene were amplified and analyzed by DNA-sequencing for the presence of mutations. A heterozygous mutation R168X in the exon 3 of the MECP2-gene was confirmed in the blood-sample of the patient. Methylation analysis of the chromosomal region 15q11-13 was performed and resulted in a normal methylation pattern.

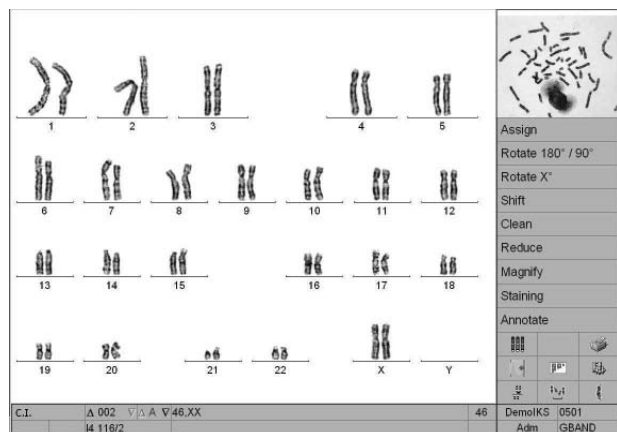


Figure 1. Normal female karyotype: 46,XX.

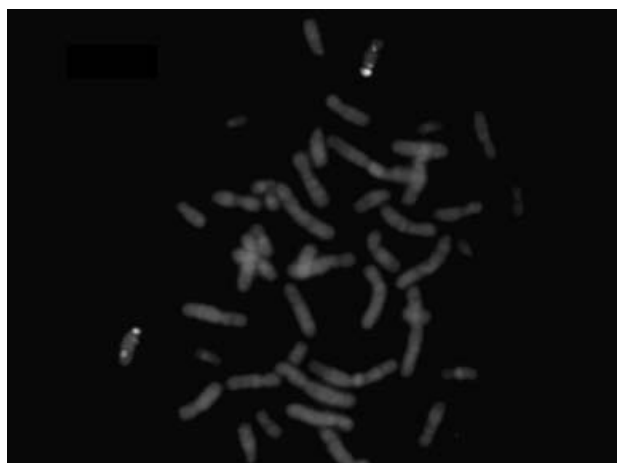


Figure 2. FISH analysis revealing the presence of signals in both chromosomes 15.

DISCUSSIONS

Genetic evaluation is an important assessment to consider when examining a patient with autism. There are several genetic syndromes that may lead to autistic symptoms. These may be caused by a chromosome abnormality or a mutant gene. The diagnosis of a genetic syndrome is important to be established for several reasons. It gives information about the cause of the autism and it may be helpful for the recommendations for the child's treatment and/or screening. Lastly, the diagnosis of a specific genetic syndrome offers information about the pattern

of inheritance, the recurrence risk for other family members, and the availability of prenatal diagnosis.

Our case, evaluated initially at 19 months, presented some clinical manifestations that are common to two genetic syndromes: Rett syndrome and Angelman syndrome. Both disorders cause a developmental ceiling with regression, are characterized by seizures, ataxia, hand stereotypes. Severe speech impairment; gait ataxia and a behavior with an inappropriate happy demeanor that includes frequent laughing, smiling, are important characteristics of Angelman syndrome, but they may be seen in Rett syndrome as well.¹⁰ Though these syndromes clinically resemble, they have different causes. Angelman syndrome is caused by a microdeletion 15q11.2-q13, while Rett syndrome is caused by mutations in the X-linked MECP2 gene, so the recurrence risks are different.^{11,12}

As the clinical differential diagnosis is quite difficult, cytogenetic analysis can be used to detect the chromosomal abnormality. However, the resolution of conventional staining method is limited; changes can only be detected when they exceed a certain size. FISH technique extends the resolution limit to 150 Kb.¹³

Genetic counseling gives the family an estimation of the recurrence risk, specific for each case. Usually Rett syndrome is caused by a "de novo" mutation. If the parents do not have the mutation the recurrence risk for a future pregnancy is considered low and for the relatives of the proband the recurrence risk is considered null. Even if Rett syndrome is considered a sporadic disorder, some familial cases were also described and the recurrence risk is high, up to 50 %. During genetic counseling it must be stated that there is a low risk of germ-line mosaicism, thus, prenatal diagnosis is recommended in all cases with a Rett syndrome daughter.

Establishing the diagnosis is important for the prognosis of the child. Many factors can influence the prognosis of the child, among them the X inactivation status in the cerebral tissue, modifier genes and type of mutation, even if there is not a strict genotype-phenotype correlation. The developmental potential is difficult to predict. Some individuals maintain some functional skills, other have severe regression. Some girls are able to retain some communicative skills with proper assistance.

It is estimated that about 60 % of the patients may retain their abilities to ambulate; the rest will be in wheel chairs. Considering the lifespan, with attention to nutritional needs and programs of physical and occupational therapies, patients may survive long into adulthood.^{14,15}

Finally, diagnosis can bring relief to parents and may help in anticipating some clinical problems.

CONCLUSIONS

Rett syndrome and Angelman syndrome are two neurodevelopmental disorders characterized by partial phenotypic overlaps. Rett syndrome is characterized by marked variability in the severity and progression of the disorder and at least in the early stages can be difficult to diagnose. Though these syndromes clinically resemble, they have different causes. Angelman syndrome is caused by a microdeletion 15q11.2-q13, but in Rett syndrome pathogenic mutations of an X-linked gene known as MECP2 have been identified in about 80% of the cases, so the recurrence risks are different.

FISH studies, usually performed in conjunction with conventional cytogenetic analysis are used to diagnose microdeletion syndromes, such as Angelman syndrome, helping the differential diagnosis.

Identification of mutations in MECP2 gene confirms the clinical diagnosis of Rett syndrome, but the pathophysiological basis is still unclear.

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