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RETT SYNDROME

Clinical and molecular characterization of two Brazilian patients

Andrea Stachon[1], Francisco Baptista Assumpção Jr[2], Salmo Raskin[3]

ABSTRACT - *Background:* Rett syndrome (RS) is recognized as a pan-ethnic condition. Since the identification of mutations in the *MECP2* gene, more patients have been diagnosed, and a broad spectrum of phenotypes has been reported. There is a lack of phenotype-genotype studies. *Objective:* To describe two cases of Brazilian patients with identified *MECP2* mutations. *Method:* We present two female Brazilian patients with RS. *Results:* Both patients presented with regression at 2-3 years of age, when stereotypic hand movements, social withdrawal and postnatal deceleration of head growth rate were observed. Both patients presented verbal communication impairment. Case 1 had loss of purposeful hand movements, and severe seizure episodes. Case 2 had milder impairment of purposeful hand movements, and no seizures. They had different mutations, D97Y and R294X, found in exons 3 and 4 of *MECP2* gene, respectively. *Conclusion:* Testing for *MECP2* mutations is important to confirm diagnosis and to establish genotype/phenotype correlations, and improve genetic counseling.

KEY WORDS: Rett syndrome, *MECP2* mutations, Brazilian cases.

Síndrome de Rett: caracterização clínica e molecular de dois casos brasileiros

RESUMO - *Contexto:* Síndrome de Rett (RS) é doença pan-étnica de fenótipo bastante variado desde que foram identificadas mutações no gene *MECP2* e um número maior de pacientes tem sido diagnosticadas. Existe uma demanda por estudos que investiguem a relação genótipo-fenotipo. *Objetivo:* Descrever dois casos brasileiros de RS com mutações identificadas. *Método:* Duas pacientes brasileiras com diagnóstico clínico-molecular de RS foram descritas buscando-se correlacionar genótipo-fenótipo. *Resultados:* Ambas pacientes apresentaram regressão aos 2-3 anos de idade, movimentos esteriotipados de mãos, retraimento social e desaceleração do crescimento encefálico. Ambas apresentaram déficit de comunicação verbal. Caso 1 também apresentou perda dos movimentos manuais intencionados e crises convulsivas graves. Caso 2 apresentou-se com comprometimento parcial dos movimentos manuais e sem história de crise convulsiva. As mutações distintas, D97Y e R294X, foram encontradas respectivamente em exons 3 e 4 do gene *MECP2.* *Conclusão:* A investigacao de mutações no gene *MECP2* é importante na confirmação diagnóstica, investigação genótipo-fenótipo, e aconselhamento genético em síndrome de Rett.

PALAVRAS-CHAVE: síndrome de Rett, *MECP2,* mutação, Brasil.

Rett syndrome (RS), a neurodevelopmental disorder predominantly affecting young females, was first described as a clinical entity by Rett in 1966[1]. However it was not internationally recognized until 1983, when Hagberg and colleagues[2] reported the clinical features of 35 young female patients with RS in Sweden, France and Portugal in the first English language publication. This syndrome is one of the most common causes of mental retardation in girls, and its prevalence rate is estimated between 1 in 10,000 and 1 in 15,000 female births[3]. Before 1999, the diagnostic criteria were formulated in the absence of a biological marker, and there was an effort to use as precise clinical criteria as possible for research purposes[4]. After an initial period of apparently normal childhood development from 6-18 months, RS patients usually experience a profound cognitive regression, accompanied by stereotypic hand movements, communication dysfunction and pervasive growth failure[4,5].

Despite the effort to delineate the clinical pres-

[1]MD, Institute of Medical Science, University of Toronto, Toronto, Ontario, Canada; [2]MD, PhD, Instituto de Psicologia e Faculdade de Medicina, Universidade de Sao Paulo SP, Brazil; [3]MD, PhD, Centro de Aconselhamento e Laboratorio Genetika, Curitiba PR, Brazil.

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Dr. Salmo Raskin - Centro de Aconselhamento e Laboratorio Genetika - Alameda Augusto Stellfeld 1516 - 80730-150 Curitiba PR - Brasil. E-mail: genetika@genetika.com.br

entation of RS, before 1999, there was no known biochemical, morphological or genetic marker for RS, so the diagnosis of any case not fulfilling all the clinical criteria was uncertain. In 1999, Amir et al.[6] identified the first mutations on *MECP2* in patients with classic and non-classic forms of RS. The *MECP2* gene is located at Xq28, and encodes the methyl-CpG-binding protein 2, which expression predominates in brain, fibroblast and lymphoblast cells[7]. There were a series of confirmatory studies[8-11] detecting mutations in the *MECP2* gene in RS patients from different countries. According to some studies[8,10], *MECP2* mutations can be found in more than 80% of females with RS. One study found that only 50% of non-familial cases had a band shift on a first screening using single strand conformation polymorphism, but subsequent sequencing the *MECP2* gene in the remainder cases detected mutations in a further six, giving a total of 72% of cases with defined MECP2 mutations[12]. A review article by the same group[13] showed that the detection rate depends upon several methodological factors, such as the accuracy of the original diagnosis and use of preliminary detection tests. Another review paper[14] indicated that only small proportions (~5-10%) of clinically well-defined RS patients do not have detectable *MECP2* mutations. Although many different mutations on the *MECP2* gene have been identified in the majority of children affected with RS, the diagnosis is still based on clinical criteria, since not all patients with the RS phenotype have *MECP2* mutation screening.

The syndrome is widely identified among different continental groups, including Brazilians. Rosemberg et al.[15] were the first to describe a girl with RS in Brazil. One year later, the first five Brazilian cases were reported by the same group[16]. Although the family that allowed the confirmation of X-linked dominant inheritance, and localization of the gene to Xq28 was originally from Brazil[7], there has been a lack of publications on the molecular genetics of Brazilian RS cases. Hence, as far as we are aware, no studies showing full sequencing of the *MECP2* gene and genotype-phenotype correlations in Brazilian patients with RS have been published. This paper describes the phenotype of two Brazilian girls with RS whose *MECP2* gene was fully sequenced, and mutations were detected.

METHOD

Subjects – Two female patients (current age 20 years and 5 years), with chronic encephalopathy and clinical suspicion of RS, were referred for molecular genetics analysis. The patients had a neurodevelopmental syndrome and no pre, peri or neonatal abnormalities. Written informed consent was obtained from the parents, including a specific consent for the publication of photos. Informed consent was obtained from the parents

Phenotypic analysis – Phenotypic data was collected from a combination of sources, including parental report, physical examination and medical records. Patients underwent detailed anamnesis, complete physical examination and laboratory investigation. Complementary analysis included electroencephalogram, brain computerized tomography scan, chromosome analysis, screening tests for inborn errors of metabolism, X-rays of the vertebral column, and sequencing analysis of the *MECP2* gene. Diagnostic criteria for classical and atypical RS, as proposed by The Rett Syndrome Diagnostic Criteria Work Group[17], were used.

DNA analysis – DNA was extracted from peripheral blood, as described previously[18]. Polymerase chain reaction (PCR) amplification of genomic DNA and automated fluorescence sequencing of the 3 coding exons of the *MECP2* gene, exons 2, 3 and 4, were done. Both strands were sequenced on an ABI automated sequencer, as previously published[19].

RESULTS

Phenotype analysis

Case 1 – A 20-year-old girl diagnosed with RS when she was 2 years old. She was born at term by C-section because the mother had two previous C-sections. Her mother reported a small amount of bleeding during the fifth week of pregnancy. There were no other complications during the prenatal period, and the perinatal period was normal. APGAR score was 9 in the first minute. Birth OFC was 34.5 cm (25th percentile). There is no history of resuscitation or neonatal distress or complications that required a neonatal unit admission. The patient had two older sisters, both unaffected. During the first 20 months of life, her psychomotor development appeared normal. Her mother reported her crawling, unsupported sitting and beginning to walk at a similar age compared with her two previous healthy sisters, and some babbling, but she spoke only one word ("goodbye"), which she used inappropriately. She lost her verbal language ability at 18 months. She remained with some mild incomprehensive sounds at 4 years old. At the time of this assessment, she was anarthric (lacking the motor ability that enables speech), and spontaneous eye-opening was preserved. She communicates with body language such as standing in front of objects to which she wants to play with. However, at 15 months of age a gradual cognitive, relational, and motor involution became noticeable. She had postnatal deceleration

of head growth, absence of expressive and comprehensive language (only babbling), social withdrawal and features of autism spectrum disorders. She lost her purposeful hand skills, and presented with hand washing stereotypic movements almost all the time. She started to hold objects at 2 months. She began transferring objects from one hand to the other, using pads of fingertips to grasp, piling blocks and scribbling on paper at the expected age. However, she lost all her hand skills at 20 months of age, when stereotypic hand movements were noticed. She started to walk at 14 months of age, and currently has difficulties walking that appear to be worsening. She was also not able to run or to walk well for long distances. At 30 months, she developed neurological episodes during sleep that were compatible with a diagnosis of epileptic tonic seizures and they later became frequent during the day. At 6-years-old, she started to have absence seizures and apnea. At 20-years-old, her pattern of seizures changed and the diagnosis of Lennox-Gastaut syndrome was confirmed by electroencephalography (EEG). Seizures were partially controlled by daily use of carbamazepine 1200 mg and valproic acid 1000 mg. Bruxism was also reported at this time. She was unable to control her sphincters. Other autonomic nervous system signs such as cyanotic lips and cold extremities were also observed concurrently with the appearance of stereotypic hand movements. There was no family history of similar cases, congenital anomalies, consanguinity, or psychiatric conditions. There was a paternal female second cousin diagnosed with Down syndrome, and a distant paternal cousin was diagnosed with autism.

During her physical examination, the patient was agitated, irritable and screaming constantly. Her height was 140 cm (below the 3rd percentile) and she weighted 40 Kg (below the 3rd percentile). Her head and neck examination showed poor eye contact and microcephaly with a head circumference of 54 cm (below the 3rd percentile). Her spine examination revealed severe scoliosis associated with kyphosis. She presented with constant hand stereotypic movements.

Imaging studies were performed. Results of her brain CT scan were normal. The EEG showed abnormal activities compatible with Lennox-Gastaut syndrome. Molecular analysis of *MECP2* gene showed a heterozygous D97Y mutation in exon 3.

Case 2 – A 5-year-old girl born at term by C-section. She has two healthy brothers and one healthy sister. There was no history of complications during the perinatal period; however her mother took heparin because of a thrombotic event during a previous pregnancy. APGAR scores were 6 in the first, and 9 in the fifth minute, respectively. There is no history of newborn intensive care unit admission. Her birth length was 49 cm (50th percentile) and OFC 34.5 cm (25th percentile). Motor development was normal for the first 15 months: she sat unsupported at 6 months, crawled at 8 months, and walked unsupported at 15 months. At 17 months the parents noticed that she was not interacting with them. She refused to be held, did not maintain eye contact, did not play, had decreased manual skills and developed stereotypical hand movements like hand mouthing. She began holding objects at 3 months and transferring them from one hand to the other at 5 months. She is still able to perform those former skills. She started using pincer grasp at 9 months and lost it at 18 months of age. She never scribbled nor pilled blocks. She cannot control her sphincters. There are neither respiratory problems nor autonomic nervous system dysfunction except for the loss of rectal and urethral sphincter control. Postnatal deceleration of her head growth rate was noted at 12 months. After the first 12 months of age, her OFC growth curve returned to the normal range. She has never spoken. At three years of age, she started babbling more frequently and using few words ("mom, done, water") after weekly speech therapy. She was able to communicate through a non-verbal language such as eye opening and turning head. She has never had any type of seizures. She had history of bruxism, which was treated with a night guard. Family history was negative for similar cases.

Current physical examination showed poor eye contact, OFC 50 cm (50th percentile) and hand stereotyped movements. She had constant rigid support in both hands in order to control her hand mouthing stereotypic movements.

Auxiliary tests were performed. Results of audiometric test at 9 months and 3 years old and fundoscopy were normal. Hormone levels including TSH, T4, cortisol, GH-RH were normal. Results of metabolic screening in urine and plasma were normal. Brain CT scan was normal. Genetic analysis revealed a heterozygous R294X mutation in exon 4 of the *MECP2* gene.

In order to facilitate the comparison between the two cases, clinical features of both cases are summarized in Table.

MUTATION ANALYSIS

The two patients had different mutations n the *MECP2* gene, D97Y and R294X. Details of the mutations found and its consequences are described in the Discussion.

Table. Summary of clinical features of two Brazilian cases with Rett syndrome.

Clinical characteristics	Case 1	Case 2
Apparently normal prenatal and perinatal period	+	+
Apparently normal development for the first six months of life	+	+
Postnatal deceleration of head growth rate	+	
Decrease (D) or loss (L) of purposeful hand skills	L	D
Communication dysfunction	+	+
Social withdrawal	+	+
Psychomotor impairment	+	
Hand stereotypes	+	+
Hand washing/wringing	+	
Hand mouthing		+
Impaired (I) or absent (A) locomotion	I	
Features of the autistic spectrum disorders: poor eye contact, severely limited socialization	+	+
Clinical seizures	+	
EEG abnormalities	+	
Use of anti-convulsive	+	

DISCUSSION

The clinical presentation of both our patients is consistent with the diagnostic criteria proposed in 1998 by the Rett Syndrome Diagnostic Criteria Workgroup[17]. Both patients presented the minimum criteria: mental deficiency, autism-like manifestations, normal early postnatal nervous system development, and communication dysfunction. Microcephaly, although common, is not an essential criterion, as observed in our case 2. As with both our patients, the disorder is usually recognized between 6 to 18 months in girls who have a plateau in developmental progress followed by a loss of purposeful hand skills that coincide with the onset of hand stereotypes, the hallmark behavioral manifestations of RS[2,4,5].

A normal perinatal history is another hallmark of RS. In mice, *MECP2* deficiency affects the stability of mature neurons but not brain development. Mice that had an *MECP2* deficiency were normal at birth (like our patients), but after a delay of several weeks, the first symptoms occurred, similar to the course of our patients[20]. Molecular genetic analysis enabled us to confirm the diagnosis of RS in the absence of some characteristic symptoms and even in the presence of uncharacteristic features, such as the absence of head growth deceleration, seizure and gait abnormalities seen in the patient described as Case 2. Our cases suggest that the diagnosis of RS should not be dismissed because some characteristic features are absent or because some uncharacteristic symptoms are present. Indeed, a study that evaluated 120 females clinically diagnosed with RS, found a broad spectrum of signs and symptoms related to their mutations in the *MECP2* gene[21].

According to the international diagnostic criteria, epilepsy is considered as secondary or supportive criteria[17]. As described in Case 1, various types of seizures usually coexist in the same patient and appear between 2 and 5 years, are not influenced by sleep or wakefulness, and their resistance to antiepileptic drugs is not uncommon[22]. Taking this clinical parameter into account, it is possible that Case 2 may develop seizures in the future.

Predictable clinical parameters are useful for counseling and therapy planning. For instance, the worry of parents of children who never had a period of regression that the regression is still to come could be relieved by the information that loss of hand function or language rarely happens after 4 years of age, according to international literature of RS. Our report of two Brazilian patients is consistent with this information. Nevertheless, according to Huppke et al.[21], there seems to be a certain period of time during which girls with RS learn to sit, walk and speak, and if these activities are not achieved during this time they will not be achieved at all. None of their patients learned to sit after the age of 30 months, speak after the age of 36 months, or walk after the age of 48 months, despite receiving physiotherapy and occupational therapy. These standards need to be double checked and better analyzed, and these limitations taken into account so that realistic goals for these therapies are set to avoid frustrating RS patients, therapists and parents.

More than 300 different causal mutations have been identified in cases of RS, including a wide range of missense, nonsense, and frameshift mutations, which can be accessed at the mutation database Rettbase[23]. Because so many mutations have been described in the *MECP2* gene thus far, the test for mutations is labor-intensive. To help determine whether the test should be performed or not, a checklist that identified RS patients with high chances of mutations was published[24]. According to their checklist score, both our patients would have been suitable for sequencing analysis of their *MECP2* gene, even

though they had never acquired language, and consequently could not fulfill one of the classical criteria (loss of language skills).

Case 1 is the third RS patient reported in the literature with the mutation D97Y. This mutation was first reported in 2001[25] as a missense mutation causing a replacement of a conserved aspartic acid for tyrosine. The mutation is located in the methyl-CpG-binding domain (MBD)[26]. The same mutation was reported one year later in a Korean study[11]. This study described relevant phenotypic variations in different persons with identical mutations in *MECP2.* Although it was not possible to establish a clear phenotype-genotype correlation in this Korean study, there was a tendency for patients with no detected *MECP2* mutation (30% in this study) to show more severe symptoms than patients with detected mutations. Their data also suggests that not only the type, but also the position of the mutation influences the phenotype. Furthermore, these findings suggest that different mechanisms are implicated in the phenotype.

The mutation found in our Case 2 patient (nonsense mutation R294X), was also previously described[27] and is the fifth most common mutation in Rett syndrome patients, with a frequency of 5,6%, according to the Rettbase[23]. It is localized in the transcriptional repression domain (TRD) and results in a premature stop codon at position 294. The TRD interacts with the co-repressor Sin3A and histone deacetylases[28]. Analyzing correlations between this *MECP2* mutations and the development of language, Yamashita et al.[27] found preserved speech in patients with R294X, which was also observed in our patient, who maintained the ability to babble.

The lack of family history for similar cases in our two patients is consistent with the fact that RS often occurs sporadically. In approximately 95% of patients *de novo* mutations in the *MECP2* gene occur; in most cases, they are of paternal origin[29].

In summary, this report describes the clinical and etiological features of two Brazilian cases of RS with mutations in the *MECP2* gene. Our analysis of *MECP2* mutations and their relation to the clinical presentation of RS has relevance for many aspects of genetic counseling and for genotype-phenotype correlations of patients with RS.

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