



Research Article

A Rett Syndrome Case with Mutation in MECP2 and Deletion of 16p11.2

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Abstract

Rett syndrome (RTT) is a rare, severe and progressive neurological disorder, which mainly affects girls. It is due mutations in *MECP2*, the most prevalent cause of classical RTT. Its common clinical manifestation is the autistic behavior that can be observed in several genetic disorders and in cases with isolated rare copy number variations (CNVs). Deletions of 16p11.2 represent one of the most recurrent, being present in about 1% of ASD cases. This report describes a girl with Rett syndrome and autism who presented both *MECP2* mutation and microdeletion of 16p11.2, with developmental delay since birth, besides dysmorphic aspects. To our knowledge, this is the first identification and characterization of a patient with these two genetic alterations. We suggest that this association might contribute to a more severe phenotype.

Keywords

Rett syndrome; *MECP2* mutation; Microdeletion of 16p11.2

Introduction

Rett syndrome (RTT) is a severe and progressive genetic disorder that affects 1/10,000 to 1/15,000 females [1]. It is characterized by an initial period of apparently normal development until six to 12 months, followed by rapid decline with loss of hand use and spoken language and the onset of stereotypic hand movements, abnormal gait and growth failure [2]. Autistic-like behaviour is observed in 30-50% of the cases [3]. RTT, previously considered a category of autism spectrum disorder (ASD), is now considered a distinct disease [4].

It is mainly caused by *de novo* mutations in Methyl-CpG-binding Protein 2 gene (*MECP2*), in a X-linked dominant inheritance pattern [5]. Loss of function of one copy of the gene leads to RTT and duplication of the gene has been found in boys with developmental delay, intellectual disability and autism [6].

ASD is a complex and heterogeneous disorder. Copy number alterations (CNVs) were shown to be an important causative mechanisms, being present in about 5-10% of cases, specially 15q11-q13.3, 16p11.2 and 22q13 [7-9]. Proportion of patients with 16p11 microdeletion and autism is high and ~1% of autistic people have this alteration. Also, the affected can present obesity, birth defects and other neurobehavioral abnormalities [10].

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Here we present an autistic girl with both *MECP2* mutation and del(16)(p11.2) with an unusual developmental and morphological phenotype in RTT, suggesting that it was the result of the association between the two events.

Materials and Methods

This study was approved by the Ethical Committee of the Institute of Biosciences, University of São Paulo (IB-USP) protocol n°121/2011. Written consent for the study was given by the proband's parents.

The patient came from an outpatient clinic for screening autistic disorder. Initially she was subjected to the laboratory protocol for etiological investigation of ASD, including karyotype and CNVs and according to her neurological phenotype, the *MECP2* gene was also investigated.

SALSA MLPA P343 Autism-1 probemix commercial kit MRC-Holland (Amsterdam, Netherlands), with probes for 15q11-q13, 16p11.2 and 22q13, was used for multiplex ligation-dependent probe amplification (MLPA) test, according to manufacturer protocol. The amplification products were identified and quantified by capillary electrophoresis on an ABI 3730 DNA analyzer (Applied Biosystems, Forster City, CA, US). The data were analyzed using GeneMarker software version 1.97 (SoftGenetics, State College, PA, US). Threshold values for the peak height ratio were set at 0.75 and 1.3 for deletions and duplications respectively which were considered as reference ratio.

Genomic DNA was extracted from peripheral white blood cells by standard procedures. The third and fourth coding exons of *MECP2* were sequenced on an ABI-3730 DNA Analyzer (Applied Biosystems, Forster City, CA, US) and analyzed by the sequencing facility of the Human Genome Research Center (http://genoma.ib.usp.br/?page_id=922). Primers and detailed PCR conditions are available under request. The sequences were aligned with normal *MECP2* reference sequence GRCh37/hg19(NM_004992.3) using Sequencher software version 5.1 (Gene Codes Corporation).

Results

The dysmorphic female patient was first evaluated in an outpatient screening clinic for individuals with autism at the age of 7 years old. She was born at 35 weeks gestation by normal vaginal delivery, with 2.0 kg, from a dizygotic twin pregnancy. The parents are non-consanguineous; mother was 24 years old and father was 25 years old at time of the infant's birth. The twin sister did not present any morphological or developmental alterations. Developmental history of the patient revealed hypotonia and severe delay from the birth, difficulties in breast feeding and deceleration of head growth.

She started to walk at the age of two years, falling very often. According the mother, autistic behavior was noticed when she was about six months old and the neuropsychomotor development was never considered as normal. At time of our investigation, she presented profound mental retardation, intellectual disability, eating difficulties, constipation, gait, truncal ataxia, gait apraxia, muscle wasting, spasticity, intermittent hyperventilation, bruxism, absence of use of the hands, cachexia, microcephaly, abnormal sleeping

pattern and hand stereotypies. We also observed broad forehead, ocular hypertelorism, flat midface, macrostomy and pointed chin. Her head circumference was 47 cm (<2nd percentile), weight was 14.7 kg (<3rd percentile) and stature was 105 cm (<3rd percentile). The echocardiogram, TSH/T4/PKU dosage, brain stem-evoked response audiometry (BERA) and GTG-band karyotype were normal. Could not perform the head magnetic resonance imaging (MRI).

At this time, autistic behaviour was diagnosed by psychiatrists based on DSM-IV criteria and specific tests. She showed impaired social interactions and language development, stereotyped and repetitive behaviour, absent eye contact and inflexible adherence to routines or rituals.

Molecular analysis revealed one heterozygous microdeletion at 16p11.2 spanning about 716Kb and encompassing *DOC2*, *HRIP3*, *MAPK3*, *MAZ*, *MVP*, *SEZ6L2* and *SPN* (Figure 1) and the mutation c.763C>T (p.Arg255X) at *MECP2*.

The father, mother and twin sister were also tested and the results were normal. Thus, both the 16p11.2 CNV and the c.763C>T at *MECP2* were interpreted as *de novo* alterations.

Discussion

Here we describe the occurrence of two *de novo* pathogenic

mutations, a nonsense mutation in *MECP2* and a microdeletion at 16p11.2 in a girl with delayed neuropsychomotor development and minor birth defects. *MECP2* regulates the expression of several important genes for brain development and plasticity, including genes already linked to autism [11]. The mutation c.763C>T (p.Arg255X) in *MECP2* is located in one of the hotspots of the gene known to be associated with a typical RTT phenotype [12]. Corresponds to the third mutation more common in RTT (6.68%) [13]. However, our patient has dysmorphic clinical signs which is not observed in RTT, and had not presented normal initial development followed by withdrawal and regression, as is common in this disease.

The 16p11.2 microdeletion had been consistently implicated in alterations in cognitive functioning and behaviour [14]. It is also associated with a unique widespread pattern of brain aberrant white matter microstructure that may underlie the impaired cognition and neuropsychiatric disorders [15]. According some authors, the full ASD penetrance in patients with 16p11.2 monosomy is not always observed and it can depend on other hit. The most common clinical manifestations are language delay and mental retardation [16-18]. Although the subjects with 16p11.2 microdeletion do not clearly constitute clinically a recognisable syndrome, certain facial features are shared among them: broad forehead, micrognathia, hypertelorism and a flat midface [19]. The micrognathia is described as one of more

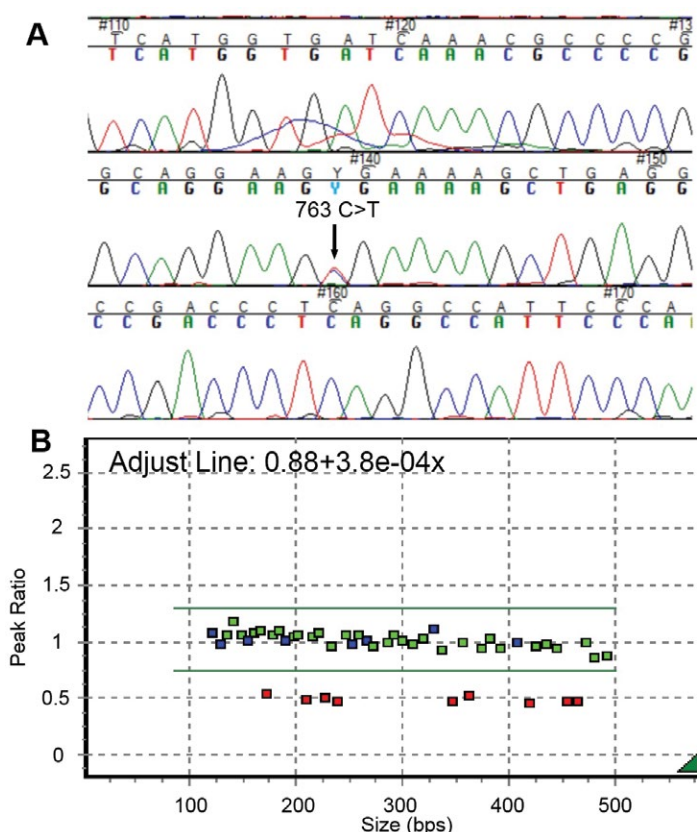


Figure 1A: DNA sequence chromatogram from RTT patient showing an heterozygous mutation in *MECP2* (c.763C>T/p.Arg255X). **1B:** MLPA peak ratio for 15q11-q13, 16p11.2 and 22q13 probes of patient.

Red: Probes with peak ratio ≤ 0.75 , corresponding to deletions of *DOC2*, *HRIP3*, *MAPK3*, *MAZ*, *MVP*, *SEZ6L2* and *SPN* genes localized on chromosomal region 16p11.2

Green: Normal probes with peak ratio ≥ 0.75 and ≤ 1.3

Blue: Control probes

common findings, but our patient does not present it. In the series of patients studied by Shinawi et al. [19], for example, macrostomia can be noted in several of them, which did not catch the attention of the

authors. Interestingly our patient has macrostomia. Seizures and obesity are also frequently described [20] which she also did not present.

Microduplications in 16p11.2 may influence or alter the expression of genes in pathways associated with ASD. This includes the *MECP2*, suggesting an overlapping mechanisms in the etiopathogenesis [21]. Further, Longo et al. [22] reported three out of 63 RTT individuals with *MECP2* mutations also were carriers of 15q11-13 rearrangements. Considering the high frequency of these CNVs (5%) in this group of RTT individuals, they suggested that the 15q rearrangements might represent one genetic determinant of the clinical variability of Rett syndrome. Therefore, it is possible that CNVs at 16p11.2 also contribute to modulate the phenotype of RTT patients, possibly contributing to severity of the case.

Genetic counselling involving two independent alterations can be challenging, particularly for the CNVs at 15q11-q13 or 16p11.2, which are associated with a still unknown penetrance and might occur as *de novo* or inherited from one of the healthy parents [17,23]. In our case, as both mutations were *de novo*, we considered the recurrence risk for RTT or ASD as negligible even if 16p11.2 CNV had been inherited from a non-penetrant parent, a risk for ASD should be considered low.

In summary, we described two rare mutational events in the same patient, which would be particularly relevant for genetic counselling purposes. Although functional performance in RTT patients can be related in general to the type of genetic defect, and that the mutation in the *MECP2* gene plays a greater role in the severity of clinical symptoms, it cannot be ruled out that the deletion in 16p11.2 also has an additive effect to the autistic phenotype in Rett syndrome. In addition, this is the case when we report these two mutations in the same individual, so we hypothesize that the deletion of 16p11.2 may be underdiagnosed in this population with Rett syndrome and deserves to be investigated in larger cases.

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Conflict of Interest

The authors declare no conflict of interest.

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