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ORIGINAL ARTICLE

Rett Syndrome: *MECP2* gene molecular analysis in Chilean patients

Síndrome de Rett: análisis molecular del gen *MECP2* en pacientes chilenas

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Abstract

Introduction: Rett syndrome (RTT) is a progressive neurological disorder characterized by regression of psychomotor development in previously healthy girls. Most cases are due to pathogenic variants in the *MECP2* gene which encodes for the methyl CpG-binding protein 2. **Objective:** To describe the frequency and type of pathogenic variants in the *MECP2* gene in Chilean female patients with clinical diagnosis of RTT. **Patients and Method:** Chilean women with clinical suspicion of RTT were invited to participate in the study. Clinical data were collected through a questionnaire. *MECP2* pathogenic variants were analyzed by Sanger sequencing method and Multiplex Ligation-dependent Probe Amplification (MLPA) was used to detect duplications or deletions. **Results:** The study included 14 patients with suspected RTT, of which eight (57%) patients had pathogenic variants. The other patients remain without molecular diagnosis. **Conclusions:** Pathogenic variants in *MECP2* are present in Chilean patients with RTT. It is likely that there are other genes or diagnoses involved in patients without *MECP2* findings. As of this study, molecular diagnosis is available in Chile.

Keywords:
Rett Syndrome;
MECP2;
Methyl-CpG-Binding Protein 2

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Introduction

Rett syndrome (RTT; MIM#312750) is a neurodevelopmental disorder that mainly affects girls and is considered virtually lethal in boys[1]. The incidence is estimated at 1 in 10,000 to 15,000 female live births[2].

RTT is diagnosed based on clinical criteria[3] and there are three clinical forms: classic or typical, variant or atypical, and mild learning disabilities, The latter is less frequent. Classical RTT is the most frequent presentation (75%)[4]. It is characterized by apparently normal development until 6 to 18 months of age, followed by a halt and subsequent regression in psychomotor development, with loss of manual skills, gait, language, and emergence of stereotypies. Atypical RTT has five possible variants: congenital, late regression, preserved speech, early epilepsy, and incomplete form, in which other genes may be involved[1-5].

Although the diagnosis is clinical, molecular study allows confirmation Pathogenic variants in *MECP2* gene are the cause of 80-90% of cases of classical RTT and about 40% of atypical RTT. Most of the variants are located in exons 3 and 4, and 99% of cases, they are *de novo* events[1-6]. The remaining cases have been associated with pathogenic variants in *CDKL5* and *FOXG1* genes, which are more frequently found in the atypical Rett Syndrome form[7-8].

MECP2 is located in Xq28 chromosome region, it is composed of four exons which encode the *Methyl-CpG-binding protein*[2,9]. This protein is involved in neuronal development and maturation, as well as in the differentiation and formation of neuronal synapses, by regulating gene expression through CpG island methylation, acting both as a repressor and transcriptional activator[10-12]. The protein has four functional domains, of which the most important are MBD (*Methyl-CpG-binding Domain*) and TRD (*Transcriptional Repression Domain*)[1,11].

In Chile, the molecular bases of Rett syndrome has not been characterized. In this study, the type and frequency of variants in *MECP2* in Chilean patients with a clinical diagnosis of RTT were analyzed.

Patients and Method

Girls, members of the *Fundación Síndrome de Rett Chile*, with suspicion or clinical diagnosis of RTT, were invited to participate prior informed consent of their parents. The study was approved by the Research Ethics Committee of the *Centro de Bioética de la Facultad de Medicina Clínica Alemana Universidad del Desarrollo*, according to the Declaration of Helsinki[13].

Clinical information was obtained through open- and closed-ended questionnaires, which included information about family history, perinatal history, and diagnostic criteria for Rett syndrome.

To detect variants and implement molecular diagnosis in the *MECP2* gene, the gene was sequenced through specific primers, designed using the Primer3 software for the coding region exons 1, 2, 3, and 4[14]. The primers were verified using SNPCheck and BLAST[15,16], and universal M13 tails were added to each primer to simplify the capillary sequencing procedure[17]. The exons were amplified by means of a touchdown program with extension at 68º-58ºC for 1 minute, using MangoTaq polymerase (Bioline, UK). The Amplified PCR products were sequenced bidirectionally with BigDye Terminator v1.1 following the manufacturer's protocol (Thermo Fischer, USA). Multiplex ligation-dependent probe amplification (MLPA) kit P245 (MRC, Holland)[18] was used to detect deletions or duplications in *MECP2*.

Results

Fifteen women between the ages of 2 and 28 years, members of the *Fundación Síndrome de Rett Chile participated in the study*. One patient was excluded because she did not meet the diagnostic criteria.

Out of the 14 patients, ten had classic RTT and four had atypical RTT characteristics. The collected information did not allow to classify these patients into the subtypes of atypical RTT. Table 1 describes the clinical manifestations.

Using Sanger sequencing, six pathogenic variants were found, five of them in patients with classic RTT presentation and one in a patient considered as atypical RTT. Five of these pathogenic variants were in exons 3 and 4, and one in exon 2. In the eight patients in whom no variant was identified, deletion and duplication analysis were performed using MLPA. With this technique, one deletion of exon 3, pathogenic variant, was detected in two additional patients: one with classical RTT and the other one with atypical form. In addition, one variant of uncertain significance (VUS) was found.

No pathogenic variants were found in the remaining five patients (Table 2).

Discussion

This is the first molecular study reported in Chile with RTT patients. Pathogenic variants were found in *MECP2* in 57% of the studied patients.

In this group of participants, the frequency of classical RTT found was approximately 70% (10 patients), similar to that described in the literature (~75%)[4], of

Table 1. Clinical and molecular characteristics of the patients

Criteria	Major criteria				Minor criteria											Exclusion criteria		Pathogenic variant
Patient	I	II	III	IV	1	2	3	4	5	6	7	8	9	10	11	i	ii	
Classic RTT																		
1	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-	-	Glu37Argfs
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	Thr158Met
3	+	+	+	+	-	-	+	+	+	-	+	-	+	-	+	-	-	Gly269Alafs
4	+	+	+	+	-	+	-	+	-	+	+	-	-	+	+	-	-	Arg270Ter
5	+	+	+	+	-	+	+	+	-	-	+	+	+	+	+	-	-	Arg306Cys
6	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	Exon 3 del
7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
8	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	-	-	-
9	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	-	-
10	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	-	-
Atypical RTT																		
11	+	+	-	+	+	+	-	+	+	+	-	+	-	+	+	-	-	Arg106Trp
12	+	+	-	+	-	+	+	-	+	+	+	+	+	+	+	-	-	Exon 3 del
13	+	-	-	+	+	+	-	-	-	-	-	-	+	+	+	-	+	-
14	-	-	+	+	+	-	+	+	+	-	+	+	-	+	+	-	+	-

Diagnostic and exclusion criteria: Major criteria: I. partial or complete loss of acquired purposeful hand skills; II. Partial or complete loss of acquired spoken language; III. Gait abnormalities; IV. Stereotypic hand movements. Supportive criteria: 1. Breathing disturbances when awake; 2. Bruxism when awake; 3. Impaired sleep pattern; 4. Abnormal muscle tone; 5. Peripheral vasomotor disturbances; 6. Scoliosis or kyphosis; 7. Growth retardation; 8. Small cold hands and feet; 9. Inappropriate laughing/screaming spells; 10. Diminished response to pain; 11. Intense eye communication - "eye pointing". Exclusion criteria for typical Rett syndrome: • Brain injury secondary to peri- or postnatal trauma, neurometabolic disease, or severe infection; • Grossly abnormal psychomotor development in the first six months of life, with early milestones not being met.

Table 2. Pathogenic variants identified in *MECP2* gene

Patient	Gene Variant	Protein effect *	Affected domain	Mutation Type
1	c.108_111delAGAA	p.Glu37Argfs **	N-terminal	Frameshift
2	c.473C>T	p.Thr158Met	MBD	Missense
3	c.806delG	p.Gly269Alafs	TRD	Frameshift lectura
4	c.808C>T	p.Arg270Ter	TRD	Nonsense
5	c.952C>T	p.Arg306Cys	TRD	Missense
6	Exon 3 deletion	Truncated protein	MBD	Large deletion
11	c.316C>T	p.Arg106Trp	MBD	Missense
12	Exon 3 deletion	Truncated protein	MBD	Large deletion

*Reference: dbSNP, ClinVar, RettBase. **Variant located in exon 2.

which 60% (6 patients) had pathogenic variants of *MECP2* and 10% (1 patient) a variant of uncertain significance (VUS). In the case of patients considered atypical RTT, 50% presented pathogenic variants in *MECP2*, which is similar to that reported in the literature[6]. The number of cases analysed was low, this is due to the low prevalence of this syndrome in the general population. In addition, the subtypes of patients with atypical RTT were not directly characterized, however, two of them have preserved speech and early psychomotor developmental delay, therefore, they may have some of these clinical variants.

Out of the eight pathogenic variants found, including deletions, four are among the most frequently described variants (Arg106Trp, Thr158Met, Arg270Ter, and Arg306Cys)[1,19]. Although pathogenic variants in *MECP2* occur *de novo*, there are more frequent variants, probably due to the presence of mutational

hotspots in this gene[20]. Deletions that include one or more exons have been observed in both patients with classic and atypical RTT[21]. This evidence suggests that it is important to use MLPA in cases where no specific variants have been found in the *MECP2* gene. 87% of the found pathogenic variants are located in the MBD and TRD, in exons 3 and 4, as described in previous studies[22,23].

An in-frame deletion of 36 nucleotides was found, c.1157_1192del36, classified as VUS. In this case, the analysis of the parents in the molecular diagnosis is important to classify this type of variants, but it has not yet been carried out in this family[24].

This study is the first to report and document pathogenic variants in *MECP2* in patients with RTT in Chile. Knowing the specific pathogenic variants confirms the diagnosis and could allow the development of future molecular therapies. In girls, where there were no findings in *MECP2*, especially the two atypical patients, it would be interesting to evaluate the other genes related to the diagnosis of RTT, *CDKL5,* and *FOXG1*, as well as to consider differential RTT diagnoses.

After this work, the molecular examination for the *MECP2* gene is available in Chile and, therefore, diagnostic certainty.

Ethical Responsibilities

Human Beings and animals protection: Disclosure the authors state that the procedures were followed according to the Declaration of Helsinki and the World Medical Association regarding human experimentation developed for the medical community.

Data confidentiality: The authors state that they have followed the protocols of their Center and Local regulations on the publication of patient data.

Rights to privacy and informed consent: The authors have obtained the informed consent of the patients and/or subjects referred to in the article. This document is in the possession of the correspondence author.

Financial Disclosure

Authors state that no economic support has been associated with the present study.

Conflicts of Interest

Authors declare no conflict of interest regarding the present study.

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