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Genetic study in a group of patients with Rett-like phenotype without MECP2 gene mutation

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ABSTRACT

Objectives: The aims of this study were (1) to identify genetic variants susceptible of causing the Rett-like phenotype in a group of Rett syndrome (RTT) or RTT-like patients without *MECP2* gene mutation; (2) to verify the presence of mutations of the *CDKL5* in some patients with suggestive phenotype.

Methods: Clinical information of 18 girls with Rett features was obtained by questionnaires and genetic analysis was performed by micro-array analysis (aCGH). The mutations found were confirmed if they were *de novo* using Polymerase Chain Reaction and aCGH for the parents. Sequencing of the *CDKL5* gene was also performed in six cases.

Results: In one case of a patient classified as classic RTT was found a mutation in *MECP2*. Mutations in the *CDKL5* gene were also found in two patients. In eight cases, copy number alterations were detected by aCGH however were not *de novo*, so that it is not clear whether they are pathogenic.

Conclusions: The clinical is the more relevant aspect in patients with RTT features and, inclusively directs by a fairly reliable way the genetic investigation. Moreover the new RTT diagnostic criteria appear to be quite trustworthy, giving a sure diagnosis, what was confirmed in case 15.

Key-Words: Rett syndrome; diagnostic criteria; *MECP2*; aCGH; *CDKL5*.

INTRODUCTION

Rett Syndrome (RTT) is a neurodevelopmental disorder that comprises the second cause of mental retardation among females [1], with a worldwide incidence between 1/10000 and 1/15000 [2] and a diagnosis rate of 1.09 per 10000 girls by the age of 12 years [3].

This syndrome affects almost exclusively females, who have an apparently normal psychomotor development until 6 – 18 months of life [4]. Thereafter, RTT patients develop a progressive syndrome characterized by cognitive and behavioral disturbances, motor impairment and autonomic dysfunction [4].

The diagnosis of RTT is based on a set of clinical criteria, irrespective of the mutation status (see **Table 1**) [5, 6].The combination of a previous personal history of developmental regression and deceleration of head growth, severe mental retardation, continuous hand stereotypies, inability to use the hands and a particularly good eye gaze raises the suspicion of diagnosis of RTT, usually when the girls are about three to five years old [7].

Table 1. Revised Diagnostic Criteria for Rett Syndrome (RTT) *in* Neul et al (2011) [6]

RTT diagnostic criteria 2010 Consider diagnosis when postnatal deceleration of head growth is observed	
Required for typical or classic RTT	**1.** A period of regression followed by recovery or stabilization **2.** All main criteria and all exclusion criteria **3.** Supportive criteria are not required, although often present in typical RTT
Required for atypical or variant RTT	**1.** A period of regression followed by recovery or stabilization **2.** At least 2 of the 4 main criteria **3.** 5 out of 11 supportive criteria
Main criteria	**1.** Partial or complete loss of acquired purposeful hand skills **2.** Partial or complete loss of acquired spoken language **3.** Gait abnormalities: Impaired (dyspraxic) or absence of ability **4.** Stereotypic hand movements such as hand wringing/squeezing, clapping/tapping, mouthing and washing/rubbing automatisms
Exclusion criteria for typical RTT	**1.** Brain injury secondary to trauma (peri- or postnatally), neurometabolic disease, or severe infection that causes neurological problems **2.** Grossly abnormal psychomotor development in first six months of life
Supportive criteria for atypical RTT	**1.** Breathing disturbances when awake **2.** Bruxism when awake **3.** Impaired sleep pattern **4.** Abnormal muscle tone **5.** Peripheral vasomotor disturbances **6.** Scolyosis/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»

RTT is caused by mutations in the methyl-CpG binding protein 2 gene (*MECP2*), located in the long arm of the X chromosome [8].

The number of patients with mutations in *MECP2* found in RTT studies varies significantly, which may be due to different inclusion criteria applied by the investigators. Therefore, *MECP2* mutations are present in classic RTT cases in about 75% to 95% [5, 9, 10, 11, 12]. In RTT variants, on the other hand, the incidence of mutations appears to be lower, these being encountered in only 20-44% of the cases [10, 11, 12, 13, 14]. However, more recently, Percy et al. [11] reported an higher percentage (73%) of *MECP2* mutation detection among atypical RTT cases. In general the mutations found in atypical RTT were identical to those found in classic RTT [15].

Recently, mutations in another X-linked gene, the cyclin dependent kinase-like 5 (*CDKL5*), located in Xp22, have been identified in patients affected by a RTT-like phenotype or with the early-onset seizures variant of RTT [16, 17, 18]. These cases showed a strikingly similar clinical course: they had seizures in the first months of life and, subsequently, they develop recognizable RTT features, such as stereotypic hand movements and hand apraxia [17].

However, there are still patients with RTT phenotype that have no known mutation, neither in the *MECP2* gene, nor in *CDKL5*.

Meanwhile, some authors have noticed that the 14q12 microdeletion was associated with a clinically recognizable phenotype that includes RTT typical features. [19, 20, 21]. After the characterization of several mutations, a candidate gene has been identified as a possible cause of a RTT-like phenotype - the forkhead box G1 (*FOXG1*) transcription factor [19]. The phenotype of *FOXG1*-mutated patients already reported, fits with the congenital variant of RTT [20], described by Rolando [22], as floppy girls retarded from the very first months of life. The *FOXG1* protein exerts a critical role in promoting neural precursor proliferation and cerebral cortex expansion, which can explain the severe microcephaly of patients with the mutation [23]. The major signs possibly indicating a *FOXG1* mutation are severe psychomotor delay with inability to walk, severe postnatal microcephaly evident before the age of four months, poor eye contact, hands and tongue stereotypies, jerky movements of limbs and corpus callosum hypoplasia [21].

The aims of this study were (1) to identify genetic variants susceptible of causing the RTT-like phenotype in a group of RTT or RTT-like patients without

MECP2 gene mutation; (2) to verify the presence of mutations in the *CDKL5* in some patients with suggestive phenotype.

SUBJECTS AND METHODS

A group (n=18) of unrelated females with RTT phenotype or with some RTT features, with ages from 16 months to 16 years (median=7years), were referred from all country (Portugal) for genetic diagnosis. Informed consent was obtained from all parents, and blood sampling was collected from children and 16 couples of parents.

Clinical information was obtained through questionnaires sent to the Neuropediatricians of the patients. The questionnaires were mainly composed of multiple choice questions and a few questions with open fields to fill, like the auxiological parameters, the age at onset of the clinical signs and the global impression of the patient (see appendix).

We gave special attention to the characteristics included in RTT criteria (**Table 1**). Besides these, other typical RTT features were examined such as deceleration of head growth, acquired microcephaly and early deviant communicative behavior (autistic features). A motor-behavioral assessment scale was used to rate the different characteristics on a 0 to 4 scale: 0= normal or never, 1= mild or rare, 2= moderate or occasional, 3= marked or frequent, and 4= very severe or constant.

Patients with epilepsy were characterized in terms of age at onset, type and drug control. In many of this patients it was also been made additional investigation (ex.: metabolic screen, cerebral MRI/CAT, karyotype, etc.) which results were also recorded.

Patients were divided in two groups according to their phenotype. Group I (GI) for those who fulfill the diagnostic RTT criteria, classical or atypical (n=12) and Group II (GII) includes individuals that are not RTT but have some RTT features, so that we considered them RTT-like (n=6).

Clinical information was treated statistically using *Excel 2007*.

Genomic DNA was extracted from peripheral blood using the Citogene® DNA isolation kit (Citomed, Portugal).

All 18 patients had already been tested for *MECP2* mutations and were negative. For six patients, classified as congenital RTT, *CDKL5* mutation was investigated by sequencing of the complete coding region (ABI Prisma from Applied Biosystems) for detection of point mutations or small insertions and deletions.

Then micro-array analysis (aCGH) was performed in the 16 patients for whom we also had blood samples from the parents. The aCGH analysis was performed on a human genome CGH Agilent 180K custom array (AMADID: 023363; Agilent, Santa Clara, CA). The array contained about 180.000 in situ synthesized 60-mer oligonucleotide probes with a resolution of approximately one probe every 17K. Hybridization and image analysis were performed using the across-array methodology described previously [24]. CGH data was analyzed using Nexus Copy Number 5.0 software with FASST Segmentation algorithm and a minimum of three probes in a region required to be considered a copy number alteration (CNV). The interpretation of CNVs was carried out based on the workflow proposed by Edelmann L et al in 2008 [25]. For each patient the total number of CNVs was listed according to the position in the chromosome and classified according to the workflow represented in **Figure 1**.

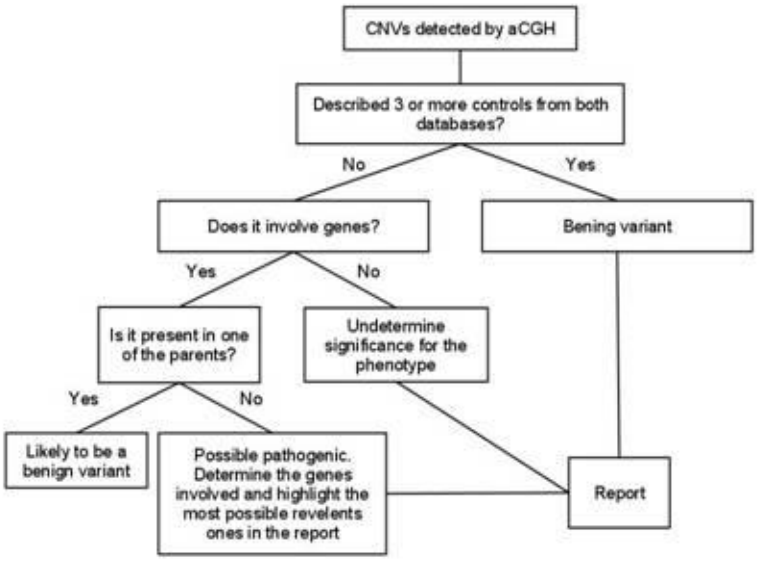

Figure 1. - General algorithm for clinical testing using array CGH (Edelmann L, Hirschhorn K, 2009).

To confirm the aCGH results was used real-time quantitative PCR (QPCR). This technique allows the detection of exon deletion or larger deletions in genes. The method involves amplifications of test exons with unknown copy number and four control samples collected by the same methods. QPCR was based on delta-delta-Ct method with 7500 Software (Applied Biosystems) [26].

As controls were used data bases (DB): an internal DB with 6000 dutch parents (presumably healthy) of mental retarded or with abnormal psicomotor development children (DuB) and a Data Base of Genomic Variants (DGV).

RESULTS

There are some missing data given because the physicians of the patients did not provide all the information.

All patients had been born after a normal pregnancy and from uneventful delivery. The median age at diagnosis was 35 months (11 months to 13 years). There is one case of parental consanguinity (first degree) and five cases (27,8%) with family history of mental retardation or autism.

In the analyzed group, ten patients fulfilled the clinical diagnostic criteria for RTT: four with the congenital form, four with early onset seizures form, one with the *forme fruste* and one classical RTT.

At birth, all individuals presented normal occipito-frontal circumference (OFC). Deceleration of head growth was verified in 53% (n=8 in 15 answers, at the median age of 6m, min=2m, max=18m), five have acquired microcephaly (29,4%) and two acquired macrocephaly. Only one case had low weight, being the majority (53,8% and 83,3% for weight and height, respectively) between P5 and P25.

Only 27,8% (n=5) were considered as having a normal psychomotor development during the first 12 months. A phase of regression/stagnation of the development was reported in 88,9% (n=16). The median age of regression/stagnation was 5 months (min=4m, max=24m).

At the age of observation, 83,3% had severe mental retardation and 55,6% had intense eye contact. Autistic behavior was noticed by parents at a median age of 7 months (min=1m, max=108m) in 83,3% (n=15).

Spoken language was acquired by some girls: simple words – 44% (median age=33m, min=12m, max=36m) and phrases – 22% (median age=4y, min=2,5y, max=5y). One patient lost the use of words at 25 months (the classic RTT patient).

Head control was reached by all patients at the median age of 4 months (min=3m, max=48m); 83,3% were able to sit at a median age of 11 months (min=6m, max=24m) but two girls had lost this capacity at 11 and 18 months. Independent gait was acquired by 61,1% (median age of acquisition=25m, min=13m, max=7,5y), one girl lost it at 10 years due to a pelvic fracture and one patient walks only with help. Among the 11 walking patients, nine (81,8%) have dyspraxic gait. Manipulation (object grasp) was achieved by 83,3% (n=15) at a median age of 13,5 months (min=2m, max=4y). Amog those with grasp, 66,7% (n=10) had purposeful prehension (median age of acquisition=36m, min=17m, max=5y), and two girls lost this capacity (one at 72m and there is no data from the other) and never reacquired.

Stereotypies were present in 94,4% (n=17) of the patients. Hand stereotypies were present in 88,2% of the patients and 23,5% show stereotypies with separated hands. The more frequent patterns was hand-washing (70,6%), hand-mouthing (23,5%), clapping (11,6%) and tapping (11,6%). More than one type of stereotypies were seen in 76,5%, and 53% show stereotypies with other body parts behind hands. Of those, trunk rocking and head rocking (17,6% each) were the more commonly reported. The frequency of stereotypies varied: 40% of the patients show them rarely, 40% occasionally and the rest frequently or constantly (10% each). This RTT feature was acquired at the median age of 30 months (min=7m, max=60m). Bruxism was present in 62,5% (n=5) of the cases for whom clinicians answered this question.

Other movement disorders besides stereotypies were present: 16,7% had tremor; 50% had dystonia (22% focal, 22% multifocal, 11% segmental and 2 cases not classified); 11% (n=2) had rigidity; no cases of athetosis were described.

A pyramidal syndrome and neurogenic atrophy were present in 38,9% of the patients and 22,2% had ataxia.

Feeding difficulties were also reported: chewing difficulties in 33,3% and regurgitation in one case (5,6%). Respiratory disturbances were described in 29,4% as periods of hyperventilation/apneia (median age=48m, min=47m, max=60m). Abdominal distension was present in 11%. Peripheral vasomotor disturbances were present in 46,8% (five girls with cold feet and in four had also small feet). Kyphoscoliosis/high kyphosis were reported in 50% (median age of diagnosis=5y, min=3y, max= 16y).

Epilepsy was reported in 55,6% (n=10), and the present type of seizures were partial crises (PC), generalized tonic-clonic (GTC) or spasms (S). One of the patients had West Syndrome at five months of age without recurrence of the seizures. The median age at onset of seizures was 6 months (min=3weeks, max=7y). All these patients were treated, 66,9% of them with more than one antiepileptic drug. Control of the seizures was achieved in 77,8% of the patients.

Sphincter control was rarely achieved (only two girls at 36 and 56 m). Moreover, 72,2% had behavioral disturbances, 61% had agitation periods, 27,8% screaming spells, and the same proportion had laughing attacks. Still, sleep disturbances are verified in 38,9% of the cases (one case of day sleep and 38,9% who wake up at night).

In the majority of the cases, no significant alterations were found at the previous investigations that were made. Except for patients 4 and 8 for whom the cerebral MRI revealed cortical atrophy, patient 14 in whom the same exam showed findings compatible with a deficit of neuronal migration and patient 13 for whom there was an abnormal cerebral CAT but no further information was available.

Considering the genetic results, there are some cases for which the aCGH did not reveal significant genomic alterations, namely for patients 1, 2, 4, 8, 9, 11 and 12.

Interestingly in patients 4 and 11 the same alteration was found by aCGH: 1p12-p11.2 deletion, this could not be confirmed in the parents but is considered benign. In **Table 3** we can see the alterations found in each patient with the respective genes involved.

Patient 3 have a chr 6p21.33 duplication; qPCR analysis performed for one of the genes involved in this alteration confirmed the CNV but revealed that it was also present in the healthy mother.

In patient 5 a 9p24.2 deletion was encountered. This alteration could not be confirmed in the parents due to difficulties in creating the probes for qPCR analysis. In patient 6 there is a 1q14 deletion and in patient 7 a 17q25.3 deletion and 1p36.31-p36.23 duplication found by aCGH, but they are also present in the parents.

For patient 10 a duplication of chromosome 10q11.23 was found, however in the DBs there are controls with smaller deletions and other almost as big as the duplication found.

In patient 13 an alteration in chr 1 (1p36-13 deletion) was found. Patient 14 had a 1p21 duplication, 3q13.31 deletion and Xp11.22 duplication; in patient 16, duplication in chr 7 was found. In these both last cases, the alterations were inherited from parents.

In patient 15 by aCGH we found a deletion of exons 1 and 2 of the MECP2 gene.

From patients proposed for CDKL5 sequencing (n=6), only two had this gene mutated: patients 17 and 18.

Table 2: Main clinical information and genetic result for each patient

Pat /G	Age	MR	DHG /age	P OFC	R/S/ age	AB/ age	IEG	G/Dys/ PGrasp	Speech level	Ster (h/o)	Other MD	B/S dist	P.Vm dist	BR Dis	Ep/Ty /age	Scol	Prev D	GR	
																		aCGH	CD KL5
1/GI	9y	Sev	N	50	Y/5m	Y/5m	N	Y/Y/Y	Phrases	Y/N	N	Y/N	N	N	Y/SW/5m	N	N	22q11.21 del	mut -
3/GI	11y	Sev	Y / NA	<5	Y/4m	Y/36m	N	Y/Y/Y	Babbling	Y/N	N	Y/N	N	N	N	Y	N	6p21.33 dup, mat	
4/GI	16y	Sev	NA	NA	Y/NA	NA	N	Y lost/NA/Y	N	Y/Y	D	Y/Y	Y	N	Y/GTC/1m	Y	EP	aCGH -	mut -
8/GI	7y	Sev	Y / 6m	<5	Y/NA	Y/11m	Y	Y+help/NAp/ Y	Phrases	Y/Y	D	N/N	Y	Y	Y/NA/84m	Y	N	aCGH -	
9/GI	12y	Sev	N	>95	Y/4m	Y/NA	Y	N/NAp /Y lost	N	Y/Y	D	N/Y	Y	N	Y/PC/7m	Y	CV	aCGH –	mut -
10/GI	16m	Sev	N	50	Y/4m	Y/4m	N	N/NAp/N	Babbling	Y/Y	N	N/N	N	N	Y/NA/4m	N	EV	10q11.23 dup	mut -
12/GI	3y	Mod	Y / 8m	5	Y/NA	Y/1m	Y	Y/Y/Y	Words	N/Y	T, D	Y/N	Y	N	Y/NA/36m	Y	FF	aCGH –	
13/GI	6y	Sev	Y / 8m	1	Y/7m	Y/8m	Y	N/NAp/N	N	Y/Y	D, R	Y/Y	Y	Y	N	Y	CV	1p36.13del	
14/GI	6y	Sev	N	2	Y/NA	Y/1m	Y	N/NAp/Y lost	N	Y/N	T, D	N/N	N	Y	Y/PC/36m	N	CV	2p21 dup, mat 3q13.31 del, pat Xq11.22 dup, pat	
15/GI	9y	Sev	Y / 18m	3	Y/24m	Y/24m	Y	Y/Y/N	Words	Y/N	D, R	Y/N	Y	N	N	Y	CR	Del exon 1 e 2 MECP2	
17/GI	2y	Sev	Y / 4m	<1	Y/4m	Y/4m	N	N/NAp/N	N	Y/N	N	Y/Y	Y	Y	Y/PC/3w (S)	Y	EV		Mut
18/GI	6y	Sev	N	4	Y/5m	Y5m	N	N/NAp/N	Babbling	N/Y	D	Y/Y	NA	N	Y/GTC + S/5m	N	EV		Mut
2/GII	6y	Mod	N	NA	N	Y/12m	Y	Y/N/Y	Phrases	Y/Y	N	N/N	N	N	N	N	N	aCGH -	
5/GII	2y	Sev	Y / 5m	<5	Y/5m	N	Y	Y/Y/Y	Words	N/N	T	Y/N	N	N	N	N	N	9p24.2 del	
6/GII	5y	Sev	N	>95	Y/NA	Y/12m	N	Y/N/N	Babbling	Y/Y	N	N/N	NA	N	N	Y	N	1q14del, pat	
7/GII	7y	Sev	NA	25	N	NA	Y	Y/Y/Y	Phrases	N/Y	D	Y/N	N	N	N	N	N	1p36.31-p36.23 dup, mat 14q24.3 dup, mat 17q25.3 del, mat 19p13.2 dup, mat	
11/GII	5y	Sev	Y / 2m	<5	Y/18m	Y/3m	N	Y/N/N	N	Y/N	N	Y/Y	N	Y	Y/NA/30m	N	CV	aCGH -	
16/GII	14y	Mod	NA	2	Y24m	Y/108m	Y	Y/N/NA	Words	Y/N	N	Y/Y	NA	N	N	N	N	7p22.2 dup, mat	

Legend: *Pat/G* - Patient/Group; *MR* – mental retardation; *DHG* – Deceleration of head growth; *P OFC* – Percentil of cephalic circumference; *R/S* –regression/stagnation; *AB* – autistic behavior; *IEG* – intense eye gaze; *G/Dys/PGrasp* - Gate/ dyspraxic gait/Porposeful grasp; *Ster (h/o)* – stereotypies (hand/other body parts); *Other MD* - other movement disorders; *B/Sdist* – behavioural/sleep disturbances; *P.Vm dist* – peripheral vasomotor disturbances; *BR Dis* – breathing disorders; *Ep/Ty/age* – Epilepsy/type/age; *Scol* – Scoliosis; *PrevD* – prevail diagnosis; *GR* – genetic results
Y- yes; *N* – no; *y* –years; *m* –months; *w* – weeks; *NA* – data non available; *NAp* – not applicable; *Mod* – moderated; *Sev* – severe; *Y lost* – acquired but then lost the capacity; + *help* – can walk with help; *T* – tremor; *D* – dystonia; *R* –rigidity; *SW* – West Syndrome; *GTC* – generalized tonic-clonic seizures; *PC* – partial seizures; *S* – spasms; *EV* – early onset seizures variant; *CR* – classical RTT; *CV* – congenital RTT variant; *FF* – *forme fruste* variant; *Dup* – duplication; *Del* – deletion; *pat* – paternal inheritance; *mat* – maternal inheritance, *dn – de novo*; *"-"* - negative; *Mut* - mutation

Table 3: Alterations found for each patient and the respective genes involved

Patient	aCGH results	Genes
1	22q11.21 deletion	*TUBA8*
3	6p21.33 duplication	*DDR1 DPCR1 GTF2H4 MUC21 SFTA2 VARS2*
10	10q11.23 duplication	*PARG*
13	1p36.13 deletion	*MFAP2 SDHB ATP12A2*
14	2p21 duplication	*COX7A2L EML4*
	3q13.31 deletion	*DRD3*
	Xq11.22 duplication	Near the *HUWE1* gene
5	9p24.2 deletion	*GLIS3*
6	1q14 deletion	*CNST SMYD3 TFB2M*
7	1p36.31-p36.23 duplication	*CAMTA1*
	17q25.3 deletion	*CCDC40 GAA*
	19p13.2 duplication	*ACP5 ZNF627 ZNF833*
16	7p22.2 duplication	*CARD11 GNA12*

DISCUSSION

We verified that many questions about clinical information were not answered by the the Neuropediatricians, even some of multiple choice. Perhaps it was due to a missing information in the clinical records. These missing data clearly influences our results.

The majority of aCGH findings were also present in the parents so they are likely to be benign.

The alteration detected in patients 4 and 11 (1p12-p11.2 deletion) was considered benign; it is also present in more cases of Portuguese mental retardation patients, thus it is likely to be a variant that is common in our population. The presence of this variant was not possible to confirm by qPCR in the parents because probes with enough specificity for these genes could not be developed (they belong to large families with lots of homologies in human genome).

In patient 1, the alteration found involves the *TUBA8* gene. This gene is expressed at a low level in the developing mouse and human brain, with the exception of the olfactory bulbs and the cerebellum. Its mutation results in polymicrogyria with optic nerve hypoplasia, by an unconfirmed mechanism [27]. Bilateral forms of polymicrogyria tend to cause more severe neurological problems. Signs and symptoms of these conditions can include recurrent seizures (epilepsy), delayed development, crossed eyes, problems with speech and swallowing, and muscle weakness or paralysis. The most severe form of the disorder, bilateral generalized polymicrogyria, affects the entire brain. This condition causes severe intellectual disability, problems with movement, and seizures that are difficult or impossible to control with medication [28].

For patient 3, the duplication found in chromossome 6 involves six genes (*DDR1, DPCR1, GTF2H4, MUC21, SFTA2, VARS2*). QPCR was performed for the

DDR1 gene and the same alteration was found in the mother. Although the qPCR was made for a small part of the gene, when searching an array alteration it is considered that the alteration found in parents has the same dimensions of the one of the child in study, so it is likely not pathogenic. The *DDR1* gene (discoidin domain receptor 1) forms a complex with E-cadherin at adhesive contacts increasing the stability of cell surface E-cadherin and promoting MDCK cell aggregation [29], moreover collagen–DDR1 signaling is essential for granule neuron axon formation and have a important role of pia in cerebellar cortex histogenesis [30]. *GTF2H4* gene seems to be significantly associated with Multiple Sclerosis in a study made by Briggs *et al.* [31], although more studies are still needed to confirm this finding.

For patient 5 a deletion in chromosome 9 involving the *GLIS3* gene was found. This alteration could not be investigated in parents to check if is inherited. Bialelic mutation of this gene had been associated with neonatal diabetes, intrauterine growth retardation, congenital hypothyroidism, facial anomalies, congenital glaucoma, hepatic fibrosis, and polycystic kidneys [32, 33]. However, this girl does not present, so far, features suggesting any of these pathologies.

In patients 6, the deletion of chromosome 1 involves the *CNST*, *SMYD3*, *FB2M* genes but was inherited from the normal father, so it may be non pathogenic.

The alterations found in patient 7 were also considered likely to be benign. The 1p36.31-p36.23 duplication affects *CAMTA1*; 17q25.3 deletion involves *CCDC40* and *GAA*; and finally 19p13.2 duplication includes *ACP5*, *ZNF627* and *ZNF833*. These three mutations were also found in the patients' mother.

Concerning case 10, it is unlikely that the deletions described in DB (several small and one with the same size of the patients' 10 duplication) disrupting the same genes are not pathogenic and duplication found in this girl is.

In the alteration detected in patient 13 (1p36.13del), there are genes involved, namely *MFAP2*, *SDHB*, *ATP12A2*. In this case we went to confirm if the *ATP12A2* gene was deleted because the remainder alteration was covered by controls with deletions in the same regions. QPCR revealed that *ATP12A2* gene was not deleted, suggesting that this too may be a benign alteration. Ramirez A, et al. [34] described a loss-of-function mutations in a predominantly neuronal P-type ATPase gene, *ATP13A2*, underlying an autosomal recessive form of early-onset parkinsonism with pyramidal degeneration and dementia (PARK9, Kufor-Rakeb syndrome). Neurodegenerative disorders such as Parkinson and Alzheimer disease cause motor and cognitive dysfunction and belong to a heterogeneous group of common and disabling disorders [34]. There are some features of these diseases that could have some correlation with RTT.

In patient 14 we found three mutations encompassing various genes (see **Table 3**). From those, the gene that is more relevant for the phenotype in study may be *DRD3* – encoding the dopamine D3 receptor. This receptor is thought to play a role in the executive function and working memory processes [35]. *DRD3* rare predominantly expressed in the nucleus accumbens, but also in the ventral tegmental area and in the amygdala [36]. Another important gene implied in these patient alterations is the *HUWE1* gene. HUWE1 is a domain E3 Ub ligase implicated in the regulation of cell proliferation, apoptosis, DNA damage response, and base excision repair [37]. This is an X-linked gene, and *huwe1*-null mouse cerebellum exhibit defective cell cycle and impairment in the differentiation of cerebellar granule neuron precursors, and disruption of the scaffold organization of radial glia. This lead to impaired granule neuron migration and heterotopic granule neuron clusters at the pial surface [38]. In fact in this patient we have information, as referred before, that there are MRI findings compatible

with a deficit of neuronal migration, which is compatible with the implications of *HUWE1* mutation, however the mutation is inherited and may also be a benign variant.

For patient 16, the duplication in chr 7 involves two genes (**Table 3**), none of them with known relevant implications in phenotype in study.

These alterations found were all considered benign. Inclusively, those found in girls 3, 6, 7, 14 and 16 are all inherited from parents. However, the possibility of these genes playing a pathogenic role in the clinical phenotype of this RTT-like and RTT atypical children cannot be ruled out definitely. It should be reinforced that these can be cases of incomplete penetrance, for example, justifying the normal parents and the affected children.

The *MECP2* mutation found in case 15 which had not been detected before, alerts us for the possibility of false negatives or any other errors that can occur during the genetic analysis conditioning the result as, for example, change of samples. This reminds us of the importance of the clinical features. In fact this girl was classified as Classic RTT, fulfilling the new diagnostic criteria for the typical presentation of this syndrome (**Table 1**). This finding corroborates that the clinic is determinant in this syndrome, making by itself the diagnosis [6].

Considering the patients for whom *CDKL5* mutation was searched, all fulfilled clinical criteria for atypical RTT (GI), all had a period of regression (between 4 and 5 months) and had an early onset of epileptic seizures (among one and seven months). However only two patients had *CDKL5* mutation. In fact, these two girls had developed early epileptic seizures (at one and five months of age), having a common issue: both have or had spasms, a seizure type that is not present in none of the other cases of this study. These two girls have poor eye contact, no grasp, no purposeful words nor phrases, difficult control epilepsy, behavior and sleep disturbances. Besides this, these

cases are in concordance with what was already described in literature: seizure onset before the regression and the development of other RTT recognizable features (deceleration of head growth, stereotypies, hand apraxia, generalized hypotonia and sleep disturbances) [16, 39, 40]. Bahi-Buisson *et al.* [39] reported that patients with *CDKL5* mutation may have phenotypes with different severity: those who do not acquire ambulation seem to have more severe microcephaly, hand apraxia, poor eye communication, bruxism and sleep disturbances; conversely, those able to walk seem to have a better eye gaze, hand use and less bruxism and sleep disturbances. In our two cases, both girls did not acquire gait yet (although they are only two and three years old, so they can develop this capacity until ten years) and have autistic traits; however only one has acquired microcephaly. Our findings corroborate that the main feature that should raise the hypothesis of a *CDKL5* mutation is, in fact, the early onset of the seizures. The epilepsy often starts within 3 months [39] and is frequently associated with West syndrome (Infantile spasms) [41, 42].

Table 4. Clinical data of patients studied compared with a group with MECP2 mutation reported by Temudo T *et al.* (2011) [12]

	MECP2 mutation (n = 59) (Temudo T, Santos M, Ramos E, et al., 2011)	**Without MECP2 mutation (n=18)**
Median age (years)*	7.6 (4.1-14.3)	7 (1.33 – 16.0)
Perinatal data (%)		
Abnormal delivery	28.8	0.0
Microcephaly at birth		
Yes	10.2	0.0
Missing information	16.9	11,1
Reported psychomotor development (%)		
Normal until 12 months of age	59.3	27.8
Stagnation / Regression	93.2	88.9
Median age of stagnation / regression (months)*	12.0 (10.0-18.0)	5.0 (4.0-24.0)
Acquisition of autistic traits	83.1	83.3
Median age of acquisition of autistic traits	18.0 (12.5-18.0)	7.0 (1.0-108.0)
Propositive manipulation	91.5	55.6
Median age of acquisition of stereotypies	20.0 (14.5-25.0)	30.0 (7.0-60.0)
Propositive words	71.2	44.0
Acquisition of independent gait	63.3	61.1
Data at observation		
Eye pointing	96.6	55.6
Mycrocephaly at time of observation	45.8	29.4
Low weight (< 5th percentile)	43.3	5.0
Low height (< 5th percentile)	55.2	0.0
Agitation	56.7	61.0
Laughing spells	69.5	27.8
Abnormal sleep pattern	55.9	38.9
Hyperpnea/Apnea	78.0	29.4
Epilepsy	57.6	55.6
controled	58.8	77.8
Vaso-motor disturbances	71.2	46.8
Neurogenic muscle atrophy	49.2	38.9
Chewing difficulties	78.0	33.3
Pyramidal signs	28.8	38.9
Ataxia	35.6	22.2
Number of stereotypies per patient*	4.00 (3.0-6.0)	2.0 (1.0-4.0)
Dystonia	64.6	50.0
Rigidity	49.2	11.0
Tremor	49.2	16.7
Ataxic/rigid gait	43.6	50
Scoliosis	72.9	50.0

*Data presented as median (25th percentile- 75th percentile)

Comparing patients involved in this study with those with MECP2 mutation reported by Temudo *et al.* [12], we can conclude that among atypical RTT or RTT-like patients there is a higher incidence of abnormal development in the first year of life. In

RTT-like patients there is no regression phase as sharp as in RTT, occurring only a period of stagnation. There are also cases with developmental delay since birth in which regression or stagnation never happen. Among the group reported in this work, the regression/stagnation occurs earlier in life comparing with *MECP2*-mutated patients.

In both groups there is a similar incidence of autistic traits, however in patients without *MECP2* mutation it is noticed earlier. In this group an inferior number of patients have propositive manipulation and propositive words, but the proportion of acquired gait is similar. Stereotypical movement onset seems to have wider age range in RTT-like group and these patients display a smaller number of stereotypie types. The incidence of epilepsy is similar in the two groups, but its control appears to be easier to reach in patients without *MECP2* mutation.

Besides this, there are other interesting differences between the two groups: patients without *MECP2* mutation have poor eye pointing, lower incidence of acquired microcephaly, laughing spells, hyperventilation/apnea, vaso-motor disturbances, neurogenic muscle atrophy, chewing difficulties, movement disorders and scoliosis.

It is easy to understand that these children have a different presentation from those with *MECP2* mutation as some of them do not have various characteristically RTT features.

The main conclusion form this study is that the clinical features are the more relevant aspect in patients with RTT features and, inclusively directs in a fairly reliable way the genetic investigation. Moreover the new RTT diagnostic criteria appear to be quite trustworthy, giving a sure diagnosis, which was confirmed in case 15.

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RESUMO

INTRODUÇÃO

O Síndrome de Rett (RTT) constitui uma doença neurológica do desenvolvimento e a segunda causa de atraso mental grave no sexo feminino, ocorrendo, mundialmente, com uma incidência de aproximada 1/10000 a 1/20000 nascimentos de crianças do sexo feminino.

As crianças afectadas têm um desenvolvimento psicomotor aparentemente normal até aos 6 – 18 meses, notando-se, posteriormente, alterações a nível do desenvolvimento psicomotor. São características deste síndrome: atraso mental grave, desaceleração do crescimento do perímetro cefálico, estereotipias manuais contínuas, incapacidade para usar as mãos voluntariamente e boa comunicação com o olhar.

O diagnóstico de RTT, habitualmente feito entre os três e os cinco anos de idade, baseia-se num conjunto de critérios clínicos (Tabela 1), sendo independentemente da presença ou não de mutação.

O RTT é causado por mutações no gene da *methyl-CpG binding protein 2* (*MECP2*), localizado no braço longo do cromossoma X. No entanto, a taxa de detecção de mutações neste gene varia de 75% a 95% dos casos de RTT clássico, por oposição a 20-73% dos casos de RTT variante.

Recentemente, identificou-se, em paciente com fenótipo de RTT – *early onset seizure*, mutações na ciclina 5 dependente de quinase (*CDKL5*). Nesta forma do síndorme, os doentes desenvolvem convulsões nos primeiros meses de vida e, só mais tardiamente surgem, características reconhecíveis de RTT.

Mutações do *forkhead box G1 (FOXG1) transcription factor* foram, também, identificadas como possíveis implicadas no fenótipo *RTT-like* em pacientes sem mutação de nenhum dos dois genes já descritos. O fenótipo dos pacientes com mutação no *FOXG1* enquadra-se na forma de RTT congénita: microcefalia pós-natal severa,

atraso psicomotor grave, contacto visual pobre, estereotipias manuais e da língua, movimentos bruscos dos membros e hipoplasia do corpo caloso.

Os objectivos deste estudo foram: (1) identificar polimorfismos genéticos susceptíveis de causar um fenótipo *RTT-like* num grupo de pacientes com RTT ou *RTT-like* sem mutação do gene *MECP2*; (2) verificar a presença de mutações de *CDKL5* em pacientes com fenótipo sugestivo.

MATERIAL E MÉTODOS

Dezoito raparigas com fenótipo de RTT ou com algumas características de RTT, entre 16 meses e 16 anos, sem mutação identificada do gene *MECP2* foram referenciadas de todo o país para estudo genético. Recolheram-se amostras sanguíneas das crianças e dos pais. Obteve-se a informação clínica através de questionários enviados aos Neuropediatras dos pacientes.

Os pacientes foram divididos em dois grupos: o grupo I (GI) inclui os doentes que preenchem os critérios diagnósticos de RTT, clássico ou atípico (n=12); e o grupo II (GII) inclui os que apresentam apenas algumas características de RTT (n=6).

Em 6 pacientes, com fenótipo de RTT congénito, pesquisou-se mutações no gene *CDKL5*.

Efectuou-se análise genética por micro-array (aCGH) e os resultados confirmados com real-time quantitative PCR (qPCR). Como controlos, usaram-se duas bases de dados (DB).

RESULTADOS

A idade mediana de diagnóstico foi de 35 meses.

No grupo analisado, dez pacientes preencheram os critérios clínicos para RTT: quatro com a forma congénita, quatro com *early onset seizures*, um com a forma fruste e um com RTT clássico.

Ao nascimento, todos tinham perímetro cefálico (PC). A desaceleração do crescimento da cabeça verificou-se em 53%, havendo cinco doentes com microcefalia adquirida (29,4%) e dois de macrocefalia. Apenas um doente apresentava baixo peso.

O desenvolvimento psicomotor nos primeiros 12 meses foi normal em apenas 27,8% dos casos. Verificou-se regressão/estagnação do desenvolvimento em 88,9%, ocorrendo numa idade mediana idades de 5 meses.

À idade de observação, 83,3% dos doentes tinham atraso mental severo e 55,6% contacto ocular intenso. Comportamento autista foi notado, numa idade mediana de 7 meses, em 83,3%.

Na amostra em estudo, 44% pronunciar palavras simples e 22% frases. Um paciente perdeu a capacidade de uso das palavras aos 25 meses.

O controlo cefálico foi atingido por todos os pacientes; 83,3% foram capazes de se sentar. A marcha independente foi adquirida por 61,1%, mas, entre estes, 81,8% têm marcha dispráxica. A capacidade de preensão de objectos foi adquirida por 83,3%, destes apenas 66,7% tinham propositada.

Estereotipias, adquiridas numa idade mediana de 30 meses, estavam presentes em 94,4% dos pacientes e as estereotipias manuais, em particular, em 88,2%. Mais de um tipo de estereotipias são observadas em 76,5% dos doentes, e 53% têm estereotipias de outras partes corporais. A frequência das estereotipias é pequena (raramente) em

40% dos pacientes e em 40% ocasionalmente, sendo constantes em apenas em 10%. Bruxismo foi referido em 62,5% dos casos.

Síndrome piramidal e atrofia neurogénica estavam presente em 38,9% dos pacientes e 22,2% tinham ataxia.

Também foram descritas dificuldades na mastigação, em 33,3%, e distúrbios respiratórios, em 29,4%. Distúrbios periféricos vasomotores estavam presentes em 46,8%. Cifoscoliose/cifose alta foram reportadas em 50% dos casos.

Epilepsia foi registada em 55,6%. A idade mediana de início das convulsões foi de 6 meses e o controlo destas foi atingido em 77,8% dos casos.

Apenas duas raparigas atingiram o controlo de esfíncteres, 72,2% tinham alterações do comportamento e 38,9% distúrbios do sono.

Da investigação adicional que foi previamente levada acabo nestes doentes, em apenas quatro casos (pacientes 4, 8, 13, 14) foram encontradas alterações, nomeadamente a nível de RMN e TAC cerebrais, com atrofia cortical e défice de migração neuronal.

Considerando os resultados genéticos, há alguns casos para os quais as aCGH não revelaram alterações genómicas significativas, nomeadamente para as pacientes 1, 2, 4, 8, 9, 11 e 12.

Para os pacientes 3, 6, 7, 14 e 16 encontraram-se alterações, representadas na Tabela 3., no entanto verificou-se que também estavam presentes nos pais das crianças. Nos outros casos (5, 10, 13), as alterações encontradas, apesar de não se ter conseguido confirmar a sua presença nos pais, foram consideradas benignas.

Na paciente 15, foi detectada uma delecção dos exões 1 2 2 do gene *MECP2* e das pacientes propostos para sequenciação do CDKL5 (n=6), apenas duas tinham este gene mutado: pacientes 17 e 18.

DISCUSSÃO

Houve várias questões acerca da clínica dos pacientes que não foram respondidaspor todos os Neuropediatras. Esta falta de dados em alguns parâmetros influenciou os resultados deste estudo.

As alterações encontradas por aCGH foram consideradas benignas, sendo que maioria destas foram herdadas dos pais.

Na paciente 1 encontrou-se uma alteração que envolve o gene *TUBA8*. Este é expresso, em baixo nível, no cérebro humano em desenvolvimento, sendo que a sua mutação está associada a uma condição conhecida como polimicrogíria.

A duplicação encontrada na paciente 3, herdada da mãe, envolve dois genes com implicações a nível do Sistema Nervoso Central: o *DDR1* comparticipação na histogénese do córtex cerebelar; e o *GTF2H4* que parece ter associação com a Esclerose Múltipla.

Na paciente 5, foi detectada uma delecção envolvendo o gene *GLIS3*, cuja mutação bialélica foi associada a diversas patologias como diabetes neonatal, hipotiroidismo, porém, esta criança não apresentou, até à data, características que sugiram qualquer uma dessas doenças.

As alterações encontradas nos paciente 6, 7, e 16 foram todas consideradas não patogénicas. A duplicação descoberta no paciente 10, será provavelmente benigna, conclusão tirada a partir da análise das alterações reportadas nas DBs.

Na criança 13, a alteração descoberta envolve a região do gene *ATP12A2*, confirmando-se por qPCR que este gene, no entanto, estava normal, sendo que as restantes regiões afectadas pela delecção estavam cobertas por controlos. Razões pelas quais esta alteração foi considerada benigna. O gene *ATP12A2* está associado a uma forma de Parkinson, doença com algumas similaridades com o RTT.

Na paciente 14, encontrámos três mutações benignas. Dos genes envolvidos, um dos mais relevantes para a patologia em estudo será o *DRD3*, que poderá participar na função executiva e nos processos da memória de trabalho. Outro importante gene implicado é o *HUWE1* - um cerebelo *Huwe1-null* exibe uma perturbação ao nível da diferenciação dos precursores dos grânulos cerebrais neuronais e uma disfunção da organização da glia, que, por sua vez, pode prejudicar a migração neuronal. Apesar deste polimorfismo parecer benigno, isto pode ser particularmente interessante, tendo em conta os achados na RMN desta paciente, compatíveis com défice de migração neuronal.

Todas estas alterações foram consideradas benignas, contudo a possibilidade destes genes desempenharem um papel patogénico no fenótipo clínico RTT-like e das crianças com RTT atípico não pode ser excluída definitivamente.

A mutação *MECP2* encontrada no caso 15 e não detectada previamente alerta-nos para a possibilidade de falsos negativos e de erros na análise genética. Além disso, reforça a fiabilidade dos critérios clínicos, que aliás indicam que esta criança é um RRT clássico.

Todas as pacientes nas quais se pesquisou a mutação *CDKL5* preenchiam os critérios clínicos para RTT atípico. Contudo, apenas duas tinham a mutação. Ambas desenvolveram convulsões epilépticas precoces e apresentavam um tipo de epilepsia que não está presente em nenhum outro caso deste estudo: espasmos. Este achado corrobora que o principal factor que nos põe na pista de uma mutação do gene *CDKL5* é o surgimento precoce de crises epilépticas (por volta dos três meses ou antes)

Comparando os doentes estudados neste trabalho com um grupo descrito por Temudo *et al* (2011) com mutação do gene *MECP2* verifica-se que a fase de regressão/estagnação ocorre mais cedo e há maior incidência de desenvolvimento anormal no primeiro ano de vida entre os pacientes com RTT atípico ou RTT-like. Nestes doentes, não há um período de regressão tão evidente como nos RTT clássico.

Em ambos os grupos, há uma incidência similar de traços autistas, contudo, em pacientes sem mutação *MECP2* são notados mais cedo. A proporção de pacientes com marcha independente e com epilepsia são similares nos dois grupos. O controlo das crises epilépticas parece mais fácil de ser conseguido nos pacientes sem mutação *MECP2*.

Os doentes com mutação *MECP2* executam uma maior variedade de estereotipias, têm melhor contacto visual, maior incidência de microcefalia adquirida, distúrbios respiratórios e vasomotores periféricos.

Assim, é mais fácil perceber que estas crianças têm uma apresentação diferente daquelas com mutação *MECP2*, não apresentando muitas características típicas de RTT.

A principal conclusão que se pode tirar deste estudo é que as características clínicas são o aspecto mais relevante no RTT, inclusivé, direccionando a investigação

genética de uma forma fidedigna. Além do mais, os novos critérios diagnósticos de RTT mostraram ser confiáveis, possibilitando um diagnóstico correcto, tal como se verificou no caso 15.

ANEXOS

Anexo I

Base de dados do Síndrome de Rett

Data do exame: …./…./……..

Idade Diagnótico de S. Rett: ……..

Consanguinidade: Sim ☐ Não ☐ 1º Grau ☐ 2º Grau ☐

Outros membros da família com S. Rett, encefalopatia epileptog. ou atraso mental

Sim ☐ Não ☐ Não sabe ☐

Gravidez: Normal ☐ Anormal ☐ Não sabe ☐

Parto: Normal ☐ Anormal ☐ Não sabe ☐

Nascido Termo: Sim ☐ Não ☐ Não sabe ☐

Peso ao nascer (g): …… **Índice Apgar** ….. / …..

Estatura ao nascer: ……

Perímetro Cefálico ao Nascer (cm): …….. Não sabe ☐

Estagnação do Crescimento Cefálico (P.C.): Sim ☐ Não ☐ Não sabe ☐

→ **Idade:** …… anos …….. meses

Microcefalia Adquirida: Sim ☐ Não ☐

Desenvolvimento psicomotor: Normal ☐ Anormal ☐

(desde nascimento até aos 12 meses)

Idade em que estagnou: ……. Meses

Perda de contacto social (Comp. Autista) **Aos** ……. meses

Controlo cefálico: …… meses

Posição Sentado: Sim ☐ Não ☐

→ **Idade em que adquiriu:** ……. meses

Senta-se e mantém: Sim ☐ Não ☐ Não sabe ☐

→ **Idade em que perdeu:** ……. meses

Marcha sem ajuda: Sim ☐ Não ☐ Com ajuda ☐

Idade em que adquiriu: ……. meses

Mantém Marcha: Sim ☐ Não ☐

Idade em que perdeu: ……. Meses

Preensão de objectos: Sim ☐ Não ☐ Não sabe ☐

Idade em que adquiriu: ……. meses

Preensão propositada: Sim ☐ Não ☐ Não sabe ☐ **Aos:** …….. meses

Idade em que perdeu: ……. meses

Aos ……. anos **readquiriu preensão de objectos**

Aos ……. anos **reaquiriu uso voluntário das mãos para** ……

Aquisição de linguagem palra: Sim ☐ Não ☐ Não sabe ☐

Idade de aquisição: ……. meses

Palavras propositivas: Sim ☐ Não ☐ Não sabe ☐ **Aos:** …….. meses

Idade em que perdeu: ……. meses

Frases propositivas: Sim ☐ Não ☐ Não sabe ☐ **Aos:** …….. meses

Idade em que perdeu: ……. meses

Aos ……. anos **recuperou:** ……..**palavras propositivas**

Aos ……. anos **recuperou:** ……..**frases propositivas**

Aos ……. anos **recuperou** …….. **canta ou repete sons**

Exame objectivo na data de observação

Impressão geral:

Perímetro cefálico actual: ……. Cm

Microcefalia adquirida: Sim ☐ Não ☐ Não sabe ☐ <3sd ☐ <4sd ☐

Peso: pPEso ; **Estatura:** pEstatura ; **Pé**: pPé

Comunicação intensa com os olhos: Sim ☐ Não ☐

Dificuldades de aprendizagem: Profundas ☐ Moderadas ☐ Ligeiras ☐

Dificuldades oro-motoras na alimentação:
Refluxo ☐ Engasgamento ☐ Botão gástrico ☐

Disfunção respiratória: Não ☐ Sim ☐ **Tipo** …… Não sabe ☐

→ **Idade em que adquiriu:** ……. anos …….. meses

Distensão abdominal: Sim ☐ Não ☐ Não sabe ☐

S. Piramidal: Sim ☐ Não ☐

Ataxia: Sim ☐ Não ☐

Estereotipias: 1, 2, 3, 4,5, 6, 7, 8, 9,10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20A, 20B, 21, 22, 23, 24, 25, 26, 27, 28, 29

→ **Idade em que adquiriu:** ……. anos ……. meses

Frequência estereotipias: 1, 2, 3, 4

Bruxismo: Sim ☐ Não ☐

Outros movimentos involuntários:

Tremor ☐ Tipo de tremor: repouso, postural, cinético

Mioclonias ☐ Descrição:

Distonia ☐ Classificação da distonia: focal, segmentar, multifocal, generalizada

Atetose ☐ Descrição:

Coreia ☐ Descrição:

Rigidez ☐ Severidade da rigidez: 1, 2, 3, 4

Distúrbios Periféricos Vasomotores: Sim ☐ Não ☐ Tipo:……

Atrofia neurogénea: Sim ☐ Não ☐

Cifoescoliose / Cifose alta: Sim ☐ Não ☐ Cirurgia ☐

Idade diagnóstico: ……. meses

A marcha é dispráxica: Sim ☐ Não ☐ **Descrição:**

Epilepsia: Sim ☐ Não ☐ Não sabe ☐ **Tipo:**

Idade em que adquiriu: ……. anos ……. meses

Tratamento: Sim ☐ Não ☐ Não sabe ☐

Controlada: Sim ☐ Não ☐ Não sabe ☐

Politerapia: Sim ☐ Não ☐ Não sabe ☐

Características EEG: Sim ☐ Não ☐ Não sabe ☐

Padrão de Sono NR ☐ ANR ☐

Controlo Esfíncter Anal: Sim ☐ Não ☐ Não sabe ☐

Idade em que adquiriu: ……. anos ……. meses

Alterações Comportamento: Agitação☐ Crises de grito☐ Crises de riso☐ Não☐

Alterações de Sono: Sono diurno ☐ Acorda de noite ☐ Não ☐

Desde a idade de: …….. até …….(meses)

Peluda: Sim ☐ Não ☐

S. Rett clinicamente diagnosticado como: Rapariga ☐ Rapaz ☐

Clássico; Congénito; Epilepsia precoce; Linguagem preservada; Regressão tardia; Forma fruste

Preencher nos S. Rett variantes e se for possível ter estes dados

Ressonância Magnética Cerebral: Normal ☐ Anormal ☐ Não executado ☐

TAC Cerebral: Normal ☐ Anormal ☐ Não executado ☐

Cariótipo: Normal ☐ Anormal ☐ Não executado ☐

Estudo metabólico: Normal ☐ Anormal ☐ Não executado ☐

Aminoácidos/plasma/LCR: Normal ☐ Anormal ☐ Não executado ☐

Ácidos orgânicos: Normal ☐ Anormal ☐ Não executado ☐

Purinas: Normal ☐ Anormal ☐ Não executado ☐

TORCH/infecções congénitas: Normal ☐ Anormal ☐ Não executado ☐

Fundo ocular: Normal ☐ Anormal ☐ Não executado ☐

Registo EEG: Normal ☐ Anormal ☐ Não executado ☐

Potenciais evocados auditivos: Normal ☐ Anormal ☐ Não executado ☐

Potenciais evocados visuais: Normal ☐ Anormal ☐ Não executado ☐

EMG/velocidade de condução: Normal ☐ Anormal ☐ Não executado ☐

Estudo Cardíaco: Normal ☐ Anormal ☐ Não executado ☐

Observações: