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Case Report

Rett Syndrome: Treatment with IGF-1, Melatonin, Blackcurrant Extracts, and Rehabilitation

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Abstract: 1) This study describes the good evolution of a 6-year-old girl genetically diagnosed with Rett syndrome (RTT), after having been treated with IGF-1, MT (MT), blackcurrant extracts (BC), and rehabilitation during 6 months. 2) The patient stopped her normal development from the first year of age. The patient showed low weight and height and met the main criteria for typical RTT. Curiously, there was pubic hair (Tanner II), very high plasma testosterone, despite low gonadotropins. No adrenal enzymatic deficits existed, and ultrasound abdominal studies were normal. Treatment consisted in IGF-1 (0.04 mg/kg/day, 5/week, sc) during 3-months and then 15-days resting, MT (50 mg/day, orally, uninterruptedly) and neurorehabilitation. The new blood tests were absolutely normal and the pubic hair disappeared. Then, a new treatment with IGF-1, MT, and BC started for another 3 months. After it, pubic Tanner stage increased to III, without a known cause. 3) The treatment followed led to clear improvements in most of the initial impairments, perhaps because of the effect of IGF-I, the antioxidant effects of MT and BC, and the increase in cyclic-glycine-proline (cGP) after BC administration. 4) A continuous treatment with IGF-1, MT and BC may recover most of the neurologic disabilities that occur in RTT.

Keywords: IGF-1; MT; Blackcurrant extracts; Oxidative stress; Mecp2; Speech therapy; Neurostimulation; cyclic glycine-proline; GPE.

1. Introduction

Rett syndrome (RTT) was first described by the Austrian doctor Andreas Rett, who gave his name to this serious neurological affectation [1], whose main clinical characteristics were later described in 1983 [2]. In 1999 RTT was postulated to be produced by a *de novo* mutation in the *MECP2* gene, a X-linked gene, that encodes methyl-CpG-binding protein 2 [3]. This protein belongs to a family of proteins [4-5], who play an important role in the organization of chromatin and the regulation of transcription after binding to methylated CpG sites [6], although Mecp2 can also bind to methylated CpA [7]. In fact, methylation of DNA can affect the structure of chromatin and lead to repression of transcription of several different genes. Therefore, mutations in the *MECP2* gene, or its deletion, impedes its physiological function as a transcriptional repressor playing a key role in the maturation of the central nervous system (CNS). Most likely, this is the reason by which Mecp2 is mainly present in postmitotic neurons, participating in the development and maintenance of synapses [8], although this protein has been found widely expressed (at different rates depending on the tissue) in most of human adult tissues [9].

Human neural development begins early in the sixth week after conception, a time in which Mecp2 is expressed at low levels [9]. The progression towards an adult brain implies a cascade of expression and sequential repression, critical and closely regulated, of many different genes that

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lead to the formation of different types of neural cells and neuronal connections, migration, and differentiation of neurons, and selective and competitive death of neurons; that favors that only specific and the most important neurons survive. All this is regulated by multiple signals and, as is logical, any alteration in the signaling sequence, produced by genetic anomalies or acquired by external factors dependent on the mother or the external environment (viruses, traumas, etc.) will condition the normal development of the brain. Another very important factor is the mechanism of synapse elimination, something physiologically occurring between early childhood and the beginning of puberty [10]; this phenomenon, known as synaptic pruning, is also influenced by environmental factors.

Although it is not the objective of this study to analyze how normal brain development occurs, this small introduction allows us to understand how the mutation of a gene, such as *MECP2*, involved in the maturation of the CNS, can produce very important neurological disorders, that in the case of RTT mainly consists of: partial or complete loss of acquired purposeful hand skills; partial or complete loss of acquired spoken language; gait abnormalities; stereotypic hand movements (clapping/tapping). These are the revised diagnostic criteria for RTT, established by RettSearch Consortium in 2010 [11]. In addition, the same RettSearch Consortium established supportive criteria for atypical RTT: breathing disturbances when awake; bruxism when awake; impaired sleep pattern; abnormal muscle tone; peripheral vasomotor disturbances; scoliosis/kyphosis; growth retardation; small cold hands and feet; inappropriate laughing/screaming spells; diminished response to pain; intense eye communication [11].

Until now, there are no specific treatments that could lead to a normalization of the clinical picture of RTT. However, it has been postulated that neurotrophic factors, as IGF-1, that play a key role in the development of the CNS, might improve the symptoms of the syndrome by promoting brain development [12-13], and increasing the low number of dendrites existing in the disease.

In this study, we analyze the evolution of a young girl with RTT, during a 6-months treatment with insulin-like-growth factor 1 (IGF-1), MT, blackcurrant extracts (given during 3-months), and rehabilitation. Although we did not achieve a complete regression of the symptoms observed upon admission, some of them disappeared or clearly decreased along the treatment carried out.

2. Case Presentation Section

2.1. Medical History

The patient was a 6-year-old, female, genetically diagnosed of Rett syndrome, who came from Bulgaria to the Foltra Medical Center to receive medical treatment. She was the second daughter of three in the family, being her sisters fully normal. There were no problems during pregnancy. Her mother did not have any toxic habit. According to the reports provided by the parents, the delivery took place by cesarean section carried out at week 38, due to previous cesarean section. Weight at birth was 2.700 kg, her size was 45 cm and the head circumference was 34.5 cm. In the family there was no medical history of interest. The development of the girl was normal during the first year of life, moment in which she began to present persistent vomiting during 6 months. This led to a series of medical studies until, through genetic tests carried out in the United Kingdom, it was diagnosed that she had Rett syndrome. The girl did not receive any kind of medical treatment. She had two sessions of physiotherapy per week, as well as equinotherapy and music therapy (one session per week in each case).

Upon admission to the Foltra Medical Centre (age 6 years), the patient had a height slightly below p3 for her age, and her body weight was also below this percentile 3. She had microcephaly; she walked alone, without any help, but without defined objectives and presenting an increase in the base of support (ataxic ambulation), with short steps. At no time did she interact with who was examining her; continuously she rubbed her hands, she did not attend orders. Nothing caught her attention. There was a convergent strabismus of the left eye. She did not speak. The joint range was

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normal in all 4 limbs, but there was kyphosis and the right scapula was higher than the left. She had drooling. She did not chew and only ate semi-solid foods. She had difficulty drinking. She did not pay attention to anything, she did not pick up objects from the ground, nor did she look into the eyes of the person speaking to her. She often tightly pressed her lips and stopped breathing. There was no sphincter control. The electrocardiogram was normal (frequency: 93 beats per minute). She had never had seizures; however, according to the parents, it was common to wake up 2-3 times during the night. There was a continuous bruxism, day and night.

During the physical examination, the existence of an incipient pubic hair, Tanner II (despite her age), attracted attention, although without a breast button or incipient hair in the armpits. In addition, there was also a discrete clitoromegaly. For these reasons, in addition to the routine blood tests that we requested before starting any medical treatment, in this case the plasma levels of gonadotrophins and sexual hormones were also assessed.

2.2. Blood analysis

Upon admission, a blood test was performed. Notably, the number of erythrocytes (5.37 x 10^6/µl), hemoglobin (14.40 g/dl) and hematocrit (42.2%) were at high levels, while leukocytes and platelets were in normal values. Plasma biochemistry was normal, excepting creatine phosphokinase (CPK: 315.2 U/L; normal values: 20 - 195 U/L). Thyroid hormones were in normal values, as it was plasma cortisol. Plasma IGF-1 and IGFBP3 values were also normal (IGF-I: 68 ng/ml; normal for her age: 55-248 ng/ml. IGFBP3: 4.8 ng/ml; normal for her age: 2.6 - 5.8 ng/ml). Surprisingly, and although the plasma levels of FSH and LH were clearly prepubertal (FSH: 0.80 mU/ml; LH: < 0.1 mU/ml), the plasma levels of testosterone were really high (3.98 ng/ml; normal values in women: < 0.45 ng/ml), equivalent to those of a postpubertal male. Plasma estradiol was lower than 5 pg/ml (lower limit of the assay).

To rule out a possible deficit of 3β-hydroxysteroid dehydrogenase or 21-hydroxylase, despite normal levels of plasma cortisol, a new blood test was performed. However, all steroids measured (dehydroepiandrosterone, dehydroepiandrosterone sulphate and androstenedione) were in normal values for the age of the patient. Therefore, we decided to perform an abdominal ultrasound study that ruled out any anomaly; specifically, the existence of any tumor mass was not detected, the ovaries were normal for age and in them there was no follicular activity.

Based on all this, we suspect that the anomalies detected (pubic hair, clitoromegaly and very high levels of testosterone in plasma) could be related to the previous sustained intake of a food containing androgens.

The blood test was repeated every 3-months until discharge, seven months after admission. Interestingly, 3 months after having started with the medical and rehabilitation treatment, the patient performed a blood test in Bulgaria, her country of origin, to which she had returned during the Christmas holidays. The results of this analysis coincided fully with those that had been made in our center a few days before those holidays. Plasma FSH had increased to 3.30 mU/ml, plasma LH was 0.10 mU/ml, and plasma testosterone was in almost undetectable values < 0.025 ng/ml. Moreover, the pubic hair had practically disappeared (Tanner stage I). This supported our idea of androgen contamination in her food; however, 3-months later, just before discharge, the situation had changed again: the pubic hair was now very black and dense (Tanner stage III, only in the pubic area), and odorous sweating existed. Plasma testosterone was 3,2 ng/ml, and FSH was 4,1 mU/ml, while plasma LH was 1,1 mU/ml. Erythrocytes, hemoglobin and hematocrit also had increased until reaching values similar to those at admission. Plasma IGF-1 was 185 ng/ml, plasma IGFBP3 was 2,7 ng/ml, and CPK had been normalized (195 U/L). Other plasma values were normal.

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142 *2.3. Medical treatments*

143 Once it was established that the testosterone values and pubertal development were not due to
144 an ovarian or adrenal pathology, or to the existence of a tumor process, the medical treatment
145 consisted in the administration of IGF-1 (Increlex, Ipsen Pharma, Barcelona, Spain; 0.04 mg/kg/day, 5
146 days/week, sc) during 3-months, 15-days resting and again the same dose during other 3-months;
147 Melatonin (MT), 50 mg/day, prepared by master formula and given orally, uninterruptedly, before
148 going to bed. In this second stage of treatment, Currantex 35M (freeze dried blackcurrant extract
149 powder, obtained from the fresh fruit of the *Ribes Nigrum L.*) was also given orally. These extracts
150 had been prepared as pills by New Zealand Pharmaceuticals NZP (Auckland, New Zealand), each
151 containing 35% Anthocyanins (35 g/100 g; 43% Delphinidim-3-rutinoside, 11%
152 Delphinidin-3-glucoside, 41% Cyanidin-3-rutinoside, 5% Cyanidin-3-glucoside). The administered
153 dose consisted of 3 pills daily for three days, then 2 pills daily for three days, and then one daily
154 uninterruptedly.

155 This medical treatment was conducted in accordance with the protocols followed in our
156 Medical Centre and in compliance with the Spanish legislation for using GH and MT "off label"
157 and the Code of Ethics of the World Medical Association (Declaration of Helsinki). Signed informed
158 consent for using GH and MT, and then Currantex 35M, was obtained from the father of the patient
159 (her legal representative). In the figures shown here, the face of the patient has been partially
160 blurred to preserve her privacy.

161 No secondary adverse effects due to the medical treatments followed was observed. At
162 discharge, both the height and the weight of the patient had increased, reaching the 5th percentile
163 (p5).

164

165 *2.4. Rehabilitation and Results*

166 Rehabilitation consisted in daily sessions (5 days/week) of Speech Therapy, Neurostimulation
167 and Occupational Therapy, Integrative and Neurosensorial stimulation (EINA), and Physiotherapy
168 (3 days/week).

169

170 1. Speech Therapy

171 Initially, the patient presented a severe affectation of expressive and receptive language. She
172 did not understand orders and she did not answer when she was called by her name. She emitted
173 primary sounds to express her mood. She did not chew or crush food, which had to be semi-solid.
174 The swallowing of liquids had to be always using a bottle and in supine position, and the drinks
175 had to be warm or hot. There was a rejection of a series of textures, flavors and temperatures. There
176 was moderate drooling.

177 The main objective in this area was focused on restoring affected stomatognathic functions
178 through passive rehabilitation techniques focused on myofunctional therapy, improving the
179 swallowing of solids and liquids and acquiring linguistic precursors.

180 At discharge, the rejection of a series of solid textures had diminished, the voluntary chewing
181 movement had begun, the labial seal had improved (so there was no more drooling); she
182 swallowed liquids at any temperature with a syringe or spoon and had increased the number of
183 babbling and guttural sounds. She even imitated the repetition, in time and type, of certain sounds
184 when she was asked to do it (for instance: O-O-O-O ..... O-O-O-O; U-U-U.....U-U-U). The bruxism
185 had disappeared.

186 These changes are shown in Table 1 and Figure 1.

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Admission	Discharge
3	1

Table 1. Thomas Stonell and Greenberg scale. Upon admission the patient presented a moderate drooling (score 3: wet lips and chin). This score was reduced until 1 at discharge (the patient never drools).

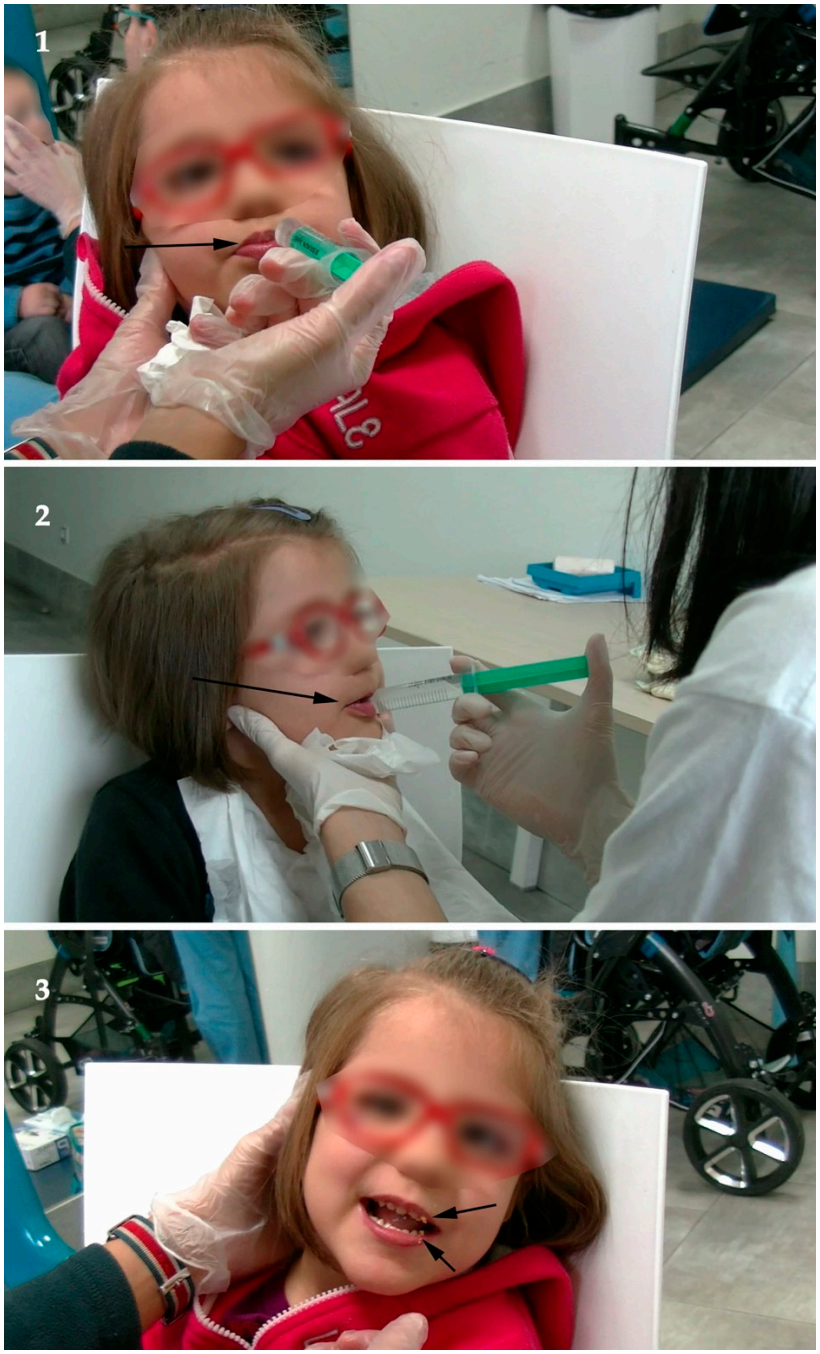

Figure 1. Speech Therapy. 1. Upon admission. Note (black arrow) how the patient seals her lips to prevent the administration of liquids with syringe. 2 and 3. Before discharge. In 2 it can be seen how the patient already accepted to drink with a syringe (black arrow). In 3 small pieces of biscuit can be seen in the mouth (black arrows) after ingesting it. Images have been blurred for avoid the identification of the patient.

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2. Neurostimulation and Occupational Therapy

The first exam of the patient showed that there was a marked alteration of the attentional capacity, there was no ocular contact or horizontal or vertical monitoring of the light. She did not interact with other children or adults, she did not recognize herself as a causal agent of events; she did not manipulate objects nor did she have acquired the permanence of them.

Therefore, the objectives in this area were directed to: foster attention, encourage communicative intentionality, explore both different objects and the environment and encourage recognition as the causal agent of events.

At discharge, in the personal and social area the patient had improved the interaction with the adult, being able to look at the face of the subject for marked periods of time and, in many cases, smiled or tried to vocalize as answers. Sometimes she was also able to react in advance to some activities she had been doing during the time of the treatment. The patient had begun to show greater awareness of her hands, using them as support when unbalanced and responding to her name when she was called. She increased his attentional capacity, observed objects and was able to follow a light in both horizontal and vertical paths for longer periods of time. In relation to fine motor skills she now keeps her hands predominantly open and began to perform ulna-palmar pressure, as well as began to touch and explore objects. As for receptive communication, she began to react to sounds that were outside her field of vision by turning her head towards the source and reacting to different types of voice. At the cognitive level, her mnesic capacity increased, as did the perceptive discrimination, reacting to new situations and beginning to visually explore her environment.

These changes are shown in Table 2 and Figure 2.

AREA	PRE-	POST-
Social/Personnel	0	2
Adaptive	1	3
Gross motor	6	8
Fine motor	0	2
TOTAL MOTOR	3	6
Receptive communication	1	5
Expressive communication	1	2
TOTAL COMMUNICATION	1	3
Cognition	1	4
TOTAL	2	3

Table 2. Scores reached in the Battelle Developmental Inventory Screening Test (BDIST) at admission (PRE-) and at discharge (POST-). Mainly note the changes observed in adaptive behavior, receptive communication and cognition.

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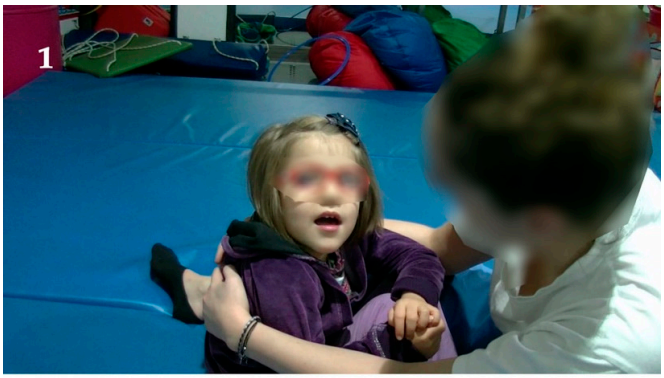

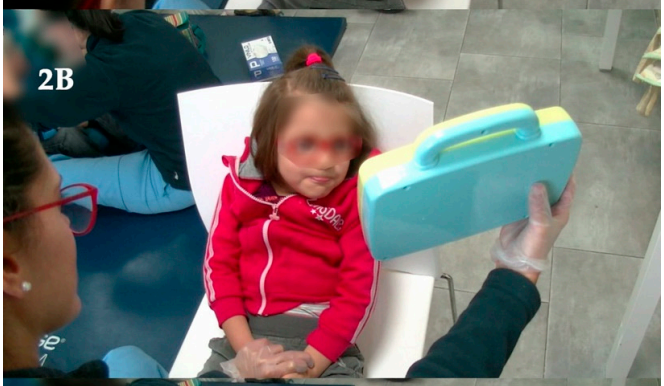

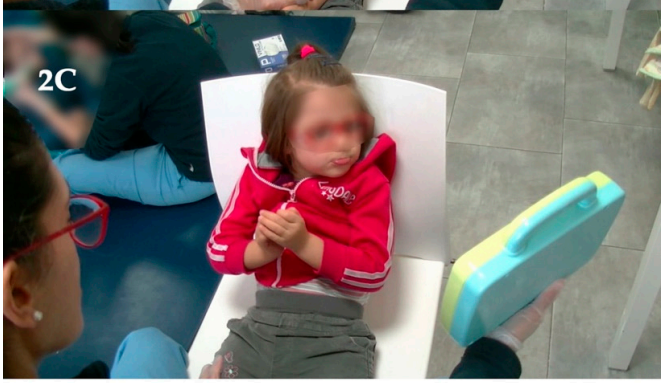

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Figure 2. Interaction with objects. 1. At admission, the patient did not look at the face of the therapist or to any other person. 2 (A to C) and 3. Before discharge she was following objects; these images show how the patient was following cartoons shown on a tablet.

3. EINA

The objective of this therapy was to stimulate the brain through the ear [14], seeking a motor and cognitive improvement and improving the results of other therapies performed by the patient.

In this case, given the condition and the lack of speech of the patient the initial and final listening tests could not be performed. Therefore, the changes that could have occurred could not be evaluated graphically. In any case, four different blocks of stimulation were carried out, with a week of interval between them. In the first two blocks, the patient listened to filtered Mozart music, Gregorian chants, and passing bands. During the third block, only Mozart music and Gregorian chants were used for brain stimulation, whereas in the fourth block the stimulation was carried out with passing bands related to balance and coordination; for this, the sound was filtered, enhancing the frequencies between 125 and 1000 Hz, so that the stimulation took place at the vestibular level.

4. Physiotherapy

At the beginning of these sessions the patient did not make postural changes. When sitting on a chair, she remained passively in it. When she was placed on the floor, she did not move or try to get up. Her defense reactions were poor, as were her straightening responses. She did not crawl, or go down or up the stairs. When she walked, she did so by increasing the base of support.

Before discharge most of these impairments had disappeared. The patient was now able to move from the supine to the prone position and vice versa. In the supine position she pushed himself to sit down; she was able to go up and down stairs and she walked with more balance, without needing to increase the base of support. Also, seeing her therapist, her face showed an expression of joy and approached her. Some of these changes are shown in Figure 3.

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Figure 3. Increased motor abilities observed at discharge. 1. The patient is now able to climb stairs. 2. The patient is able to get on a bed with the help of her hands.

3. Discussion

This study analyzes the evolution of a six years old girl that fulfilled the main criteria required for typical Rett syndrome diagnosis, established in 2010 by the RettSearch Consortium [11]. Moreover, the patient also showed many of the supportive criteria also established by this group [11], and had a genetic confirmation of the existence of a mutation in the *MECP2* gene, responsible for the development of this syndrome, present in 1:10,000 females and the second known cause of important intellectual disabilities in them. We treated her with IGF-1, MT, blackcurrant extracts and rehabilitation during a short period of time (six months of treatment with IGF-1 and MT, and then blackcurrant extracts were also added), and the results obtained have to be considered as good in terms of some improvements in her disabilities.

As stated in the Introduction, Mecp2 plays a key role in the development of the brain, mainly acting on the formation of synaptic connections. Therefore, any mutation in the gene encoding the expression of this protein has to be expected to produce devastating disorders, due to the lack of synapses and their dysfunction, as it occurs in RTT [15]. Studies in mice have shown that these deficits can be recovered, at least partially, if there is a postnatal activation of Mecp2 [16, 17]. This has been shown to occur with the administration of IGF-1, who recovers dendritic spines in mice deficient in Mecp2, although this treatment has to begin very early, when the phenotype of the syndrome is still not too severe [18]. The positive effects of IGF-1 administration in RTT have been confirmed in mouse models [19, 20], and in human patients [12, 13, 21-23], corroborating our results in this study.

Many reasons can explain why IGF-1 exerts positive effects on RTT. In rats, IGF-1 is widely expressed in many brain areas, although its expression soon decreases after birth [24], most likely in a way similar to what happens in humans; in fact, IGF-1 expression has been found in neural stem cells derived from fetal human forebrains [25]. In contrast, the receptor of IGF-1 (IGF-1R) maintains a stable expression in the brain throughout life [26], perhaps to bind to the IGF-I that, coming from the plasma, reaches the brain crossing the blood-brain barrier [27]. The binding of IGF-1 to its receptor, a membrane-bound tyrosine kinase, induces the activation of the PI3K/Akt and MAPK (Mitogen-Activated Protein Kinases, also known as ERK: Extracellular signal-regulated kinases) signaling pathways, who are also transduction pathways of BDNF (Brain Derived Nerve Factor) [28], a peptide with important neurotrophic activities, among them inducing synaptic plasticity, which, interestingly, is transcriptionally regulated by Mecp2 [29]. This explains why *MECP2* gene deficiencies or mutations lead to a marked downregulation of BDNF expression in the brain of RTT mice or humans [30, 31], but also why IGF-1 administration may contribute to improve some of the neurological abnormalities observed in RTT. Moreover, plasma levels of IGF-1 levels have been found to be decreased in 8 of 23 RTT patients [32], and the GH/IGF-1 axis has been shown to function abnormally in RTT [33]. However, treating RTT patients with IGF-1 implies its administration during the whole life, which is risky because of its mitogenic potential.

In 1989 it was identified that in the brain, IGF-1 suffered a specific proteolytic cleavage leading to the generation of two fragments: des-N-(1-3)-IGF-1, and the N-terminal tripeptide Gly-Pro-Glu (GPE) [34]. This mechanism of breakage of IGF-1 was further identified in the serum of the rat [35], and GPE was also detected in human urine [36]. Soon it was discovered that this peptide was not only a mere product of degradation of IGF-1, but it exerted important activities at brain level (see [37] for review), acting as neuromodulator, neuroprotector and even inducing the proliferation and migration of neural stem cells [37]. In addition, our data demonstrated that GPE signals through activation of the PI3K/Akt and MAPK (ERK) pathways, as do IGF-1 and BDNF [37]. However, the short life of GPE in plasma when administered intravenously limits its therapeutic use. Currently, a chemically modified form of GPE, called Trofinetide (formerly called NNZ-2566), makes the peptide suitable for oral administration, with a longer half-life in plasma and easy passage to the brain.

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These are the reasons by which this modified GPE is being used in clinical trials in RTT human patients with significantly promising results.

Another metabolite of IGF-1, possibly derived from GPE, is the dipeptide cyclic-glycine-proline (cGP). This small peptide has been shown to act by modulating the bioavailability of IGF-1, that is the amount of free IGF-1, the biologically active fraction. cGP regulates the binding of IGF-1 to its binding proteins, particularly to IGFBP3, therefore normalizing IGF-1 function under pathological conditions [38, 39]. In this sense, it has been shown that cGP increases the activity of IGF-1 when it is low (as occurs in the RTT brain), but inhibits it when IGF-1 is in high values; this may explain why IGF-1 can show effects as different as improving the recovery of brain lesions in rats and decreasing or inhibiting the growth of some tumors in mice [38], but also why cGP improves memory in adult rats [40].

On the other hand, it has been demonstrated that oxidative stress (OS) exists in RTT patients [41], although there are doubts about whether this increase in OS occurs due to the disease or is the factor responsible for it [42]. Animal studies showed a clear increase in the production of harmful reactive oxygen species in the brain of mice deficient in MeCP2, apparently produced by a defective functioning of complex II of the mitochondrial respiratory chain, that could be recovered with the administration of a bacterial protein, CNF1, which had also been shown to improve the affected neural phenotype of these animals [43]. Even though it has been suggested that RTT could be a mitochondrial disease, the discovery of mutations of the MECP2 gene showed that they precede mitochondrial dysfunction [44], although it is clear that OS plays a very important role in expression and severity of the symptoms in the disease; therefore, treating mitochondrial dysfunction or the use of reactive oxygen species scavengers may be useful in RTT patients [44].

This was one of the reasons why we used MT in our patient. Seminal studies demonstrated that MT exerts potent scavenging effects on toxic reactive oxygen species [45, 46]; in addition, this hormone has anti-inflammatory properties and is a mitochondrial protector, besides playing many other important roles in the body [47-50]. In RTT, as in many other central nervous system pathologies, neuroinflammation is the most frequent finding. It mainly occurs as a result of overproduction of inflammatory cytokines leading to an increased stress on brain cells and a strong activation of microglia. Microglia plays a prominent role in maintaining synapsis and pruning dendrites, but these abilities are lost when it is over-activated. Therefore, the administration of MT must have played an important role in the positive evolution of our patient RTT, added to the administration of IGF-1 (which, as previously indicated, is deficient in the brain of RTT).

Interestingly, the positive evolution of the patient was even greater when we were able to give her blackcurrant extracts in addition to IGF-1 and MT. It is not the objective of this study to analyze the properties of these anthocyanins, but studies carried out years ago show that they act as strong antioxidants, anti-inflammatories and anti-neurodegeneration. Of interest here is that the extracts we have used have recently been described that act through the mitochondrial pathways PI3k/Akt and MAPK, at least in cancer cell line cultures [51]. In addition, it has been demonstrated that the administration of 28 days of black currant anthocyanin increased the concentration of cGP in cerebrospinal fluid samples collected from 11 patients with Parkinson's disease; this increase in cerebral cGP correlated with plasma cGP concentration and plasma cGP / IGF-1 ratio, without modifying the concentration of IGF-1 and IGFBPs in both plasma and cerebrospinal fluid [52]. Therefore, it is likely that the blackcurrant extracts contributed significantly, acting as antioxidants, anti-inflammatory and increasing the bioavailability of cerebral IGF-1 (free IGF-1), to the additional positive evolution of our patient with RTT.

At this point, it seems to be possible to explain the unexpected and discrepant findings observed in the gonadotropic axis and pubertal development of the patient throughout the treatment, a finding not described before.

Although we did not evaluate the secretion of growth hormone (GH), presumably this was elevated, despite her low height, before commencing the treatment with IGF-1 and MT. Plasma IGF-1 values were low and this leads to increased GH release [53]. GH exerts an important stimulatory role on the gonads [54]; it is, therefore, possible that a high secretion of GH has

contributed to the high levels of testosterone initially observed and the incipient pubertal development, despite the low levels of FSH and LH. When the treatment began and IGF-1 was given, plasma IGF-1 values increased leading to diminished GH secretion. When the patient started treatment and IGF-1 was administered, the plasma IGF-1 values increased, which had to lead to a decrease in GH secretion. This may explain why pubic hair disappeared and plasma testosterone levels were undetectable in the next two analysis carried out. However, shortly after the administration of blackcurrant extracts, pubertal development reappeared and with greater intensity. This may depend on increased IGF-1 bioavailability, due to increased plasma cGP [52]; IGF-1 seems to act synergistically with GH, or independently of it in gonadal functions [53]. This explanation is merely speculative, but it merits further studies.

In summary, treatments with IGF-1, MT and blackcurrant extracts are useful for improving the neurologic disabilities existing in girls with Rett syndrome. Since extracts of blackcurrant increase the levels of cGP, the mitogenic potential of IGF-1 can be counteracted, so that treatments with this hormone can be prolonged longer. There is the need to investigate whether the androgenic abnormalities observed in our patient and their changes could have been produced by any of the treatments given.

A limitation of this study is the fact that the girl was used to listen Bulgarian language, very different from the Spanish used by her therapists, as well as the fact that she could only receive treatment for six months due to the work of her parents in their country. Currently, she is still treated with MT and blackcurrant extracts there, but without IGF-1, waiting for the European approval of GPE.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, J.D.; Methodology, O.D., M.C., N.C., A.D., D.L., C.G. and J.D; Validation, J.D.; Formal Analysis, O.D., M.C., N.C., A.D., D.L., C.G. and J.D.; Investigation, J.D.; Writing-Review & Editing, J.D.; Supervision, J.D.;

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