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Opinion Article

Treatment for Anxiety and Future Treatments in Rett Syndrome

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Abstract

Rett syndrome (RTT) is a neurodevelopmental disorder caused by mutations in the X-linked MECP2 gene that mainly affects females and few males. The prevalence of RS is estimated around 1/15,000 of newborn females. The progressive appearance of the signs and symptoms of RTT has been classified in four stages. The evolution of the clinical symptomatology and severity is individual depending on the age of onset and type of mutation. Anxiety-like behavior is frequent at all ages and is a significant parental concern in RTT. Anxiety leads to more irritability, stereotypes, repetitive rocking and great discomfort for the RTT patients. There is no curative treatment nor one specific therapy for anxiety. Alterations of hypothalamic pituitary adrenal (HPA) axis function seem to have a very important role in anxiety. Our objective is to look through all pharmacotherapy, supplementary diets existing up to-day that have been investigated or used. We review all the trials published, drugs that can be useful, reducing anxiety and added our own experience. Gene therapy can be a curative treatment and 2 trials will start very soon. Our aim is to help clinicians and caregivers to understand and give more quality of live to patients with this rare disease.

Key words: *Rett Syndrome; Anxiety; Modo Disorders; GABA A1R; KCC2/NKCC1 Ratio Therapies*

Introduction

Beyond the classic form of RTT, a number of atypical forms with different degrees of severity have been described: the Zappella variant (known as the preserved speech variant) the form fruste, the infantile seizure onset type with mutations also in chromosome X, and the congenital form with mutations in *FOXG1* [1] Besides the *MECP2* gene, additional genes have been associated with the RTT phenotype [2, 3]. It's still an object a discussion if *CDKL5* and *FOXG1*mutations are responsible for atypical RTT or are really different diseases. Rett syndrome (RTT) is a neurodevelopmental disorder caused by mutations in the X-linked *MECP2* gene that mainly affects females (OMIM#312750) [4] and few males have been also published with Rett syndrome [5, 6].

MECP2, which binds methylated CpGs, is a chromatin-associated protein that can both activate and repress transcription. It is required for maturation of neurons and is developmentally regulated.

The prevalence of RS is estimated to vary around 1/15,000 of newborn females in all ethnic groups) [7]. Over 95 % of individuals with RTT have mutations in methyl-CpG-binding protein (*MECP2*), a gene on Xq28, which encodes a transcriptional regulator [8]. MeCP2 is a protein involved in synaptic development and maintenance [8]. The diagnosis of RTT is clinical; approximately 98 % individuals who meet diagnostic criteria for typical RTT have a MECP2 mutation, while about 80 % of those meeting criteria for atypical RTT carry a *MECP2* mutation [9]. However, 3–5 % of individuals meeting diagnostic criteria for RTT do not have a *MECP2* mutation [2]. While the overall clinical presentation varies across individuals, some mutations have been associated with a more severe phenotype, including p.Arg106Trp, p.Thr158Met, p.Arg168X, p.Arg255X, p.Arg270X, and large deletions. However, other mutations have a relatively milder phenotype, such as p. Arg133Cys, p. Arg294X, p. Arg306Cys, and 3′ truncations [10, 11]. Although RTT is not considered a progressive disorder, the clinical severity of most mutations increases with age.

The most common clinical manifestation is recognized by apparently normal psychomotor devel-

opment until 6 – 18 months of age, followed by severe regression in which hand skills and expressive language are lost [12]. The typical accompanying symptoms are hand-washing stereotypies and gait apraxia. Seizures, scoliosis, and breathing disturbances such as periodic apnea during wakefulness may become evident [13]. Gross motor capabilities are influenced by the type of mutation present in the *MECP2* gene and are generally poorer in those requiring surgical correction for scoliosis. Autonomic dysfunction which may manifest as hyperventilation or breath holding, or peripheral vasomotor disturbances is a well-recognized but poorly understood feature of Rett syndrome. Abnormal or disturbed sleep patterns and behavioral issues also appeared to persist. Similarly, gastrointestinal issues such as constipation, reflux, and feeding difficulties remained prevalent [14] and may contribute to poor growth.

The progressive appearance of the signs and symptoms of RTT has been classified in four stages, where the evolution of the clinical symptomatology and severity is individual depending on the age of onset and type of mutation. The first stage arises between 6 to 18 months, where the first symptoms of neurological stagnation begin to manifest: low weight, cranial growth slowdown, decrease in communication and eye contact, difficulty in crawling and wavy movement of the hands. In stage II, starting at 18 months, patients show a regression in previously acquired skills: autistic behaviors, stereotyped hand movements, loss of motor skills and the normal sleep pattern, apraxia, ataxia, and intellectual disability come up. Stage III appear around three years of life and is characterized by a pseudo-stabilization of the disorder. Autistic manifestations regress and visual communication skills are restored. More pronounced epileptic seizures emerge. Finally, stage IV generally appears in late childhood where late motor impairment occurs. Patients present with severe scoliosis, cachexia, dystonia, loss of gait, and a reduction in seizures.

Expert Opinion

Anxiety like behaviors and stereotypes appear to be very common, which typically have a cognitive impairment and limited expressive communication. They are a prevalent and disabling component of the RTT phenotype. Among these problematic behaviors, anxiety is a prominent symptom. Anxiety-like behavior is frequent at all ages and is a significant parental concern in RTT. Girls' stereotypes were described in 99.5% of classical Rett syndrome been clapping/tapping more often than wringing/washing and has a relation with their state of anxiety. Alterations of hypothalamic pituitary adrenal (HPA) axis function seem to have a very important role in the onset of anxiety. The most widely accepted theory is that early stressful life events may provoke alterations of the stress response and thus of the HPA axis. activation and the sustained increase of cortisol levels [15].

In fact, physical and psychological stress experiences activate the hypothalamic pituitary adrenal (HPA) axis through the secretion of corticotropin releasing hormone (CRH) and arginine vasopressin (AVP) by the parvocellular neurons of the paraventricular nucleus of the hypothalamus. These neuropeptides activate the synthesis and the release of adrenocorticotropin hormone (ACTH) from the anterior pituitary, which successively stimulates the adrenal cortex to synthesize glucocorticoids, i.e., cortisol in humans. Cortisol influences several physiological processes and the synthesis of neurotrophic factors, with effects on mood and behavior [16].

Moreover, the unremitting activation of the HPA axis is supposed to be mediated by changes in the sensitivity, or in the number, of CRH and/or glucocorticoid receptors of the hippocampus, limbic system and cortical levels (brain is associated with anxiety disorders [17]. Also, the emotional state of the patients with RTT can be further exacerbated by the physical difficulties observed, for example, epileptic seizures can lead to a heightened emotional state and often leads to anxiety but can also include screaming, labile mood and uncontrollable crying. Regarding lifespan, emotion and behavior are thought to change during the time course of RTT. Behavioral dysregulation can present with increased stereotypies, repetitive rocking, scratching, self-injurious or self-stimulatory behavior, and agitation. In RTT, evidence has shown that individuals with milder mutations are more likely to exhibit mood disturbances such as anxiety/inappropriate fear in comparison to individuals with more severe mutations who were

less likely to report such difficulties [18].

Actually, some approaches have been investigated: pharmacological treatment aimed to restore signaling pathway activity, supplementary diets and reinforcement therapies, and genetic therapy that re-establish *Mecp2* gene expression. Antianxiety agents such as benzodiazepines have been relatively understudied in ASD. They are clinically useful at times in the treatment of anxiety, particularly as add-on to an SSRI. However, behavioral side effects such as numbness, or crying, and irritability limit their use and taking care if they are taking antiepileptic drugs. On the other hand, a few drops of benzodiazepines in the mouth help to resolve the anxiety crisis and also seizures if they are coming up, as we have used with good results.

Typical Neuroleptics and antipsychotics, as first-generation antipsychotics, especially haloperidol, thioridazine, and trifluoperazine have been used with poor results and several secondary effects. Tricyclic antidepressants (TCA), such as desipramine, imipramine, nortriptyline, amitriptyline as well as bupropion, a dopamine reuptake blocker, have been used to treat patients with autism for comorbid anxiety, obsessive–compulsive disorder, but clinical response and side effect severity appear less favorable and warrant caution as they can produce seizures. Other ways despite producing more frequent and severe adverse effects than, for example, fluoxetine or fluvoxamine, in healthy patients without seizures and with normal cardiac function, TCAs are generally safe and well tolerated. The predominantly 5-HT reuptake blocker most studied in ASD is clomipramine, Nortriptyline, and Tianeptine did not show benefit in the trials with placebo and most patients left the assay due to poor efficacy and side effects but initial dose of 10mg and raised to 20mg in children with more than 5 years old should be assayed in Rett patients.

Mecasermin, recombinant human IGF-1, was given to 12 Rett girls from 2-10 years of age) to evaluate safety, tolerability, pharmacokinetics, and preliminary assessments of efficacy. The latter include evaluations of neurobehavioral measures, exploratory biomarkers, and their corresponding pharmacodynamics data. In the first 4 weeks, escalated doses from twice daily (BID) injections of 40 μg/kg the first week, 80 μg/kg the second week, and 120 μg/kg in the 2º and 3º week, followed by 20-week open-label extension (OLE). The aim was to assess CNS penetration and PK profile of IGF-1, and to test the feasibility of automated cardiorespiratory measures. They conclude that long term treatment shall be necessary, considering IGF-1's likely effects on synaptic maturation and maintenance. The effect of IGF-1 was mild and selective, influencing certain cardiorespiratory and neurobehavioral features of RTT [19].

Several double-blind, placebo-controlled, parallel-group trials have been done at different ages adolescent and adult with RTT. Different doses (35mg/kg,70mg/kg, 200mg/kg and 1gr BID) have been given in oral solution in several trials. Diarrhea (and vomits are frequent adverse effects). [20]. In phase 3 study in a cohort of girls and women 5–20 years of age, Rett syndrome females received twice-daily oral Trofinetide (n = 93) or placebo (n = 94) for 12 weeks. Adverse events included diarrhea (80.6% for Trofinetide versus 19.1% for placebo), which was mostly mild to moderate in severity.

Efficacy end points included the Rett Syndrome Behavior Questionnaire (RSBQ), a caregiver assessment of core RTT symptoms (coprimary), the Clinical Global Impression–Improvement (CGI-I) scale (coprimary), and the Communication and Symbolic Behavior Scales Developmental Profile Infant-Toddler Checklist-Social (CSBS-DP-IT Social) composite score (key secondary). And adverse events. Trofinetide demonstrated a statistically significant improvement over placebo for both the coprimary and key secondary efficacy endpoints. Treatment with Trofinetide improved key symptoms of the syndrome from the perspective of both the caregiver (RSBQ) and clinician (CGI-I). Recently Trofinetide, under the brand name Daybue, has been given approval by the FDA as a treatment for RTT in patients aged 2 years and above in a study with 1380 RTT patients followed during years were evaluated on severity of mood disorders patients been anxiety one of the frequent problems. The most commonly used drugs were escitalopram, sertraline, fluoxetine, and buspirone. The proportion of patients on anxiolytic treatment was higher when they were over 18 years of age than for pediatric participants [21].

The most successful management of anxiety and mood behaviors is the use of selective serotonin reuptake inhibitors (SSRIs). SSRIs are thought to modulate the HPA axis and its downstream regulatory targets. Thus, SSRIs by adjusting serotonin levels might improve mood in RTT patients. The classical SSRI fluoxetine, might be beneficial for the management of RTT symptoms by acting through different mechanisms. SSRIs antidepressants, often in association with the 5-HT1A agonist buspirone or similar compounds do not show immediate anxiolytic effects, but have a delayed onset of action, the full clinical effectiveness may require 2–4 weeks to manifest itself.

There are some uncertainties about the effect of Antipsychotics (D2 blockers) on anxiety, probably they slightly reduce obsessions, compulsions and disruptive behaviors and can be effective in reducing irritability, and in producing a slight decrease in social withdrawal, stereotypies. The long-term use of risperidone, although generally well-tolerated, has been associated with an increase in plasma glucose, insulin, prolactin and leptin proportional to the dosage; the major problems often resulted from continuous weight gain over time and judged excessive, but in some RTT girls can be useful.

Epilepsy has been reported in 60%- 80% of MECP2 patients. Seizures start in the II and III stage of the disease. In MECP2, 60% the patients had a mutation, in one of the eight most common mutations (R106W, R133C, T158M, R168X, R255X, R270X, R294X, R160C), 9% had C-terminal deletions and 8,5% had large deletions [22]. Mutations T158M and R106W are the most common and can be very resistant to treatment. Antiepileptic drugs when they control epilepsy reduce the effect on anxiety specially when the girls are aware that a seizure is coming. Topamax at low doses has been useful in some girls reducing stereotypes and anxiety in our personal experience

In our study Rett syndrome patients showed 90% epilepsy with significantly reduced levels of KCC2 and KCC2/NKCC1 ratio in the cerebrospinal fluid of Rett patients suggesting an immature pattern of GABAergic neurotransmission in RTT patients, by revealing a dysregulation on the KCC2/NKCC1 ratio (the two major contributors to intracellular chloride concentration) showing an imbalance between excitatory and inhibitory synaptic events. The results could bring light to new therapeutic approaches, particularly through the pharmacological manipulation of the cation chloride cotransporters [23]. The expression of the gamma-aminobutyric acid (GABA) receptor-related synaptic proteins from the mouse and in postmortem brain biopsies of two Rett patients was studied, specifically revealing the GABA A1R subunit overexpression. The identification of the molecular changes along with the Rett syndrome prodromic stages strongly endorses the importance of time frame when addressing this disease, supporting the need for a neurotransmission-targeted early therapeutic intervention. The development of novel drugs enhancing GABA-A1 R function (for potential use in the initial clinical stages) and devoid of side effects are required, for an early intervention of Rett syndrome [24].

Ketogenic diet with a proposed mechanism of action that increases in the synthesis of GABA and decreases in the synthesis of excitatory neurotransmitters, such as glutamate has been assayed with poor results and only some benefit on reducing seizures in epileptic RTT patients resistant to antiepileptic drugs [25]. Ginkgo biloba extract has been reported to affect the neurotransmitter system and to have antioxidant properties A trial of the effectiveness of Ginkgo biloba as an adjunctive agent to risperidone was given. One group received risperidone plus Ginko T and the other received risperidone plus placebo. Adding Ginkgo biloba to risperidone did not affect the treatment outcome of children with autistic spectrum disorder, some families RTT have tried also with no results [26].

Carnitine and CoQ have also been given with no effects on behaviors and anxiety disorders in RTT. Cognitive stimulation has potential for brain activation in individuals with Rett syndrome and conducted a cognitive task using an eye-tracker with simultaneous EEG recordings **w**as designed to evaluate access and choice skills this facilitates communication and reduces anxiety in these patients with no verbal language, [27, 28] but futures studies have to evaluate all brain activity and metabolites and evaluate pharmacological therapies. Genetic therapy to re-establish *Mecp2* gene

expression recent advances shed light on the promises of gene replacement therapy with new vectors designed to control the levels of MeCP2 expression. New developments in DNA/RNA editing approaches or reactivation of the silenced X chromosome open the possibility to re-express the native MeCP2 locus at endogenous levels [29].

Current strategies still face limitations in transduction efficiency. Host immune responses against the AAV vector and transgene have made their widespread application not easy. Multiple factors, including vector design, dose, and route of administration, contribute to the overall immunogenicity of AAVs. Gene expression over time is very important. Immune responses against the AAV capsid and transgene involve initial innate detection. Various strategies such as extensive immunosuppression, capsid engineering, tolerance induction, etc. are being developed to circumvent the immune-mediated toxicities associated with AAV. The innate and adaptive immune response against AAVs, highlighting the challenges and potential strategies to mitigate these responses, thereby enhancing the therapeutic potential of AAV gene therapy will be possible in the near future [30].

Actually, Aysha gene Therapies has announced the initiation of clinical development of gene therapy with T TSHA-102 in adult women with RTT in Canada. Another trial will be done by Neurogene they also have announced NGN-41, a new gene therapy candidate for RTT and wants to initiate a Phase 1/2 trial to assess the safety, tolerability and efficacy of NGN-401 in female pediatric patients with RTT. The open-label, single-arm, multicenter clinical trial will evaluate a single dose of NGN-401 delivered using a one-time ICV procedure. It is an adeno-associated virus (AAV) gene therapy investigational product that is the first to deliver the full-length human *MECP2* gene, under the control of Neurogene's EXACT self-contained gene regulation technology. EXACT enables therapeutic levels of the protein MeCP2 while avoiding overexpression and related toxicities, as it could produce a duplication of MECP2 that is also another rare disease if the dose is not exact [31].

Our aim in this work is to help clinicians and caregivers to understand take care and give more quality of live to patients with this rare disease.

Declaration of Interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

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