**New insights into the mechanism of action of trofinetide: An exploratory in silico study**

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**Abstract**

**Keywords:**

**Introduction**

Rett syndrome (RTT) is a rare and complex genetic neurodevelopmental disorder that primarily affects girls, with an estimated prevalence of 1 in 10,000 to 15,000 live births [1]. It is characterized by a period of apparently normal development, followed by a progressive regression of motor, social, and communication skills. Critical symptoms of the disease include the loss of voluntary use of the hands and the appearance of repetitive movements, gait disturbances, severe cognitive impairment, respiratory irregularities, seizures, dysautonomia, and metabolic alterations such as dyslipidemia, insulin resistance, or mitochondrial dysfunction [2–4]. These manifestations reflect a multisystemic affectation that impacts both the central nervous system as well as the endocrine and bioenergetic systems. The course of the disease leads to a progressive deterioration that compromises the quality of life of patients, who require permanent medical attention and specialized care [5]. Although life expectancy can extend into adulthood, it varies depending on the phenotype, severity of symptoms, and quality of medical management [6].

The main cause of RTT is the presence of mutations in the MECP2 gene, located on the X chromosome, which encodes the methyl CpG-binding protein 2, a transcription regulator essential for neuronal maturation and synaptic plasticity [7]. There are also atypical variants of the syndrome that occur less frequently and are due to mutations in the CDKL5 or FOXG1 genes [8]. The disease usually appears between 6 and 18 months of age and causes lifelong disability, with no curative treatment currently available. Since in its early stages, symptoms can be confused with disorders such as autism, early diagnosis represents a major clinical challenge.

Until recently, the therapeutic approach to RTT was limited to disease control through the use of drugs and physical, occupational, and speech therapies. These actions are primarily aimed at mitigating the most disabling clinical manifestations, such as motor disorders, epileptic seizures, respiratory, digestive, and autonomic disorders, as well as behavioral problems. In this context, the use of anticonvulsants, anxiolytics, serotonergic modulators, beta-blockers, as well as interdisciplinary functional support strategies, is common [9].

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| --- | --- | --- | --- |
| Protein | | Acronym | Relevance in RTT |
|  |  | GAT1 |  |
|  | GABAB |  |
|  | GABAA |  |
|  | CHRM1 |  |
|  | NMDAr |  |
|  | D2Dr |  |
|  | 5HT2A |  |
|  | A2AR |  |
|  | NKCC1 |  |
|  | hSERT |  |
|  | AChE |  |
|  | MAOB |  |
|  | COMT |  |
|  | AMPA |  |
|  | σ1R |  |
|  |  | GSK3β |  |
|  | PPARγ |  |
|  | ProdH |  |
|  | HMGCR | Modula síntesis de colesterol; su inhibición mejora síntomas motores y longevidad en modelos Mecp2. |
|  | PTP1B |  |
|  |  | XO |  |
|  | SOD |  |
|  | COX‑2 |  |
|  | P2X7 |  |
|  | nNOS |  |
|  |  | UGT2B15 |  |
|  | OATP1B1 |  |
|  | P450 |  |

In 2023, the FDA approved the drug Trofinetide, marking a milestone by becoming the first targeted therapy for this disease [10]. Trofinetide is a peptide derived from the N-terminus of insulin-like growth factor 1 (IGF-1) and was designed to cross the blood-brain barrier and partially restore impaired neuronal functions in patients with MECP2 deficiency. Clinical trial results have shown that its administration produces significant improvements in some symptoms of the disease, such as non-verbal communication, social interaction, breathing pattern, sleep, and reduction of stereotyped movements [11]. However, its use is frequently associated with side effects, particularly diarrhea, decreased appetite, vomiting, and weight loss, which may require dose adjustment or discontinuation of treatment in severe cases [12].

Although no information has been reported that allows a precise elucidation of the mechanism of action of Trofinetide, available preclinical and clinical studies suggest that it modulates different convergent processes in RTT. It is known that the administration of the drug promotes the reduction of neuroinflammation, the activation of microglia, the formation of new neuronal connections and the restoration of dendritic structure [13,14]. These effects could be related to the signaling pathways regulated by IGF-1, although a specific molecular target that explains its action has not been identified [15].

The present study aims to explore, using structure-based computational tools, the potential interactions of trofinetide with a set of proteins relevant to the pathophysiology of Rett syndrome, to generate mechanistic hypotheses about its multitarget mode of action. A systematic approach is proposed that analyzes the coupling of trofinetide with proteins classified into four functional groups (Table 1).

**Methodology**

**Results**

**Conclusions**

**References**

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