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Motor behavioural tests for phenotype evaluation of mouse models of ataxia: the case of Marinesco-Sjögren Syndrome

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Abstract. Ataxias are a clinically relevant group of neurological diseases characterized by impaired motor coordination that affects both static and dynamic control of body movements. Among the autosomal recessive ataxias there is Marinesco-Sjogren syndrome (MSS). The main clinical signs of MSS are incoordination with cerebellar atrophy, and myopathy associated to hypotonia. Mouse models of MSS (e.g. woozy mouse) closely mimic human pathology and are widely used to study this disease. In fact, the woozy mouse was helpful in understanding that molecular alteration occurs before clinical signs. To data, several motor behavioural tests have been developed including rotarod, beam walking, and grip-strength. Some of these tests were effective to reveal MSS ataxia only at specific stages of the disease. To achieve these results, it is important to apply the most appropriate motor behavioural tests that are able to sensitively assess the specific phenotype. However, the administration of additional motor tests would be useful to better define the coordination and motor problems in MSS. Cognitive tests should also be considered to examine whether the woozy model has mental retardation. In conclusion, motor and cognitive tests are essential for the assessment of disease stage and future therapies in ataxias, including MSS.

Keywords: ataxia, Marinesco-Sjögren Syndrome, SIL1, mouse model, motor test.

INTRODUCTION

The cerebellum is the region of the brain responsible for motor movement coordination, balance and walking. Impairments of this area lead to deficits in controlling voluntary muscle activity, maintaining posture and motor learning (Jimsheleishvili and Dididze, 2023). These symptoms can be enclosed in the term "ataxia", which refers to a physical finding due to cerebellar dysfunction.

Ataxias are classified mainly in hereditary and acquired. Autosomal dominant ataxias are identified as spinocerebellar ataxias (SCA), while autosomal recessive cerebellar ataxias (ARCAs) are classified according to the presence and the severity of sensory neuropathy (Kuo, 2019).

ARCAs are characterized by high genetic heterogeneity and variable phenotypes (Beaudin et al., 2017). Among them, Friedreich's ataxia is the most common form, and it is used as referring point. Indeed, it is possible to distinguish between Friedreich's ataxia like disorders, with and without cerebellar atrophy, and early-onset ataxias with cerebellar atrophy, such as ataxia telangiectasia, and Marinesco-Sjögren Syndrome (MSS) (Fogel and Perlman, 2007).

MSS is characterized by cerebellar ataxia due to degeneration of Purkinje cells, associated with myopathy and congenital cataracts. Symptomatology may include also mental retardation, short stature, skeletal deformities, hypergonadotropic hypogonadism and intention tremor (Anna-Kaisa Anttonen, 2006).

SIL1 gene mutation is the major cause of MSS (Anttonen et al., 2005; Senderek et al., 2005). This gene encodes the endoplasmic reticulum (ER) cochaperone Sil1, required for ADP-ATP exchange from the chaperone BiP, which in turn is able to release folded proteins.

Non-functional Sil1 protein is responsible of the accumulation of unfolded proteins into the ER, thus triggering the activation of the unfolded protein response (UPR), which contributes to neurodegeneration and myopathy (Figure 1) (Chiesa and Sallese, 2020; Potenza et al., 2021; Restelli et al., 2019). Both cell lines and mouse models (e.g. woozy mice) carrying the SIL1 mutation are useful for studying MSS (Roos et al., 2014; Ruggieri and Sallese, 2022; Zhao et al., 2005).

In this work we focus our attention on the study of mouse models and in particular on motor behavioural tests, which are considered powerful tools in the case of neurological diseases (Brooks and Dunnett, 2009). A more comprehensive characterization of the motor functions of woozy mouse may be useful to better understand MSS and its progression, and to evaluate the efficacy of potential therapeutic treatments.
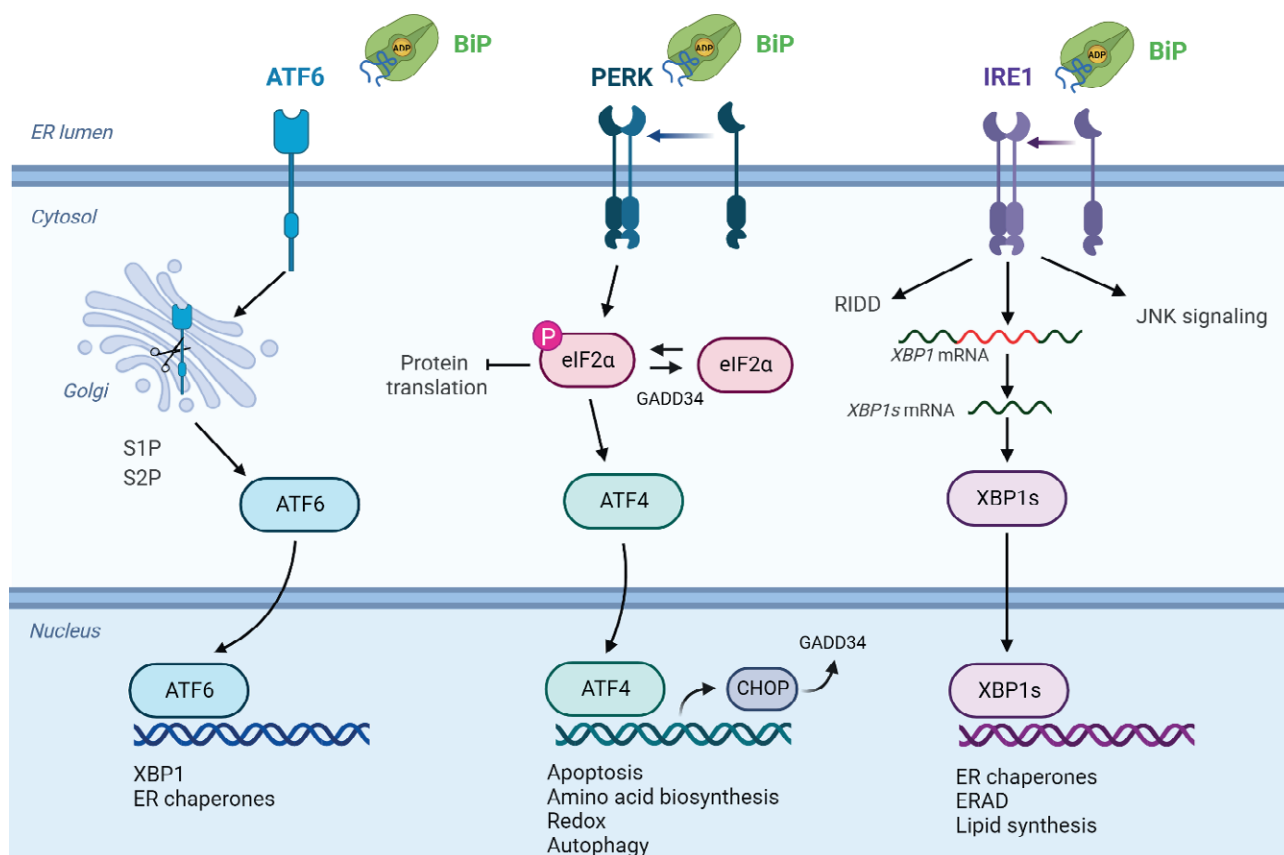

Figure 1. Unfolded protein response is a cellular defense mechanism whose purpose is to reestablish the normal protein homeostasis in the ER, through the activation of three ER transmembrane sensors: inositol-requiring enzyme 1 (IRE1), activating transcription factor 6 (ATF6), and protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK). Their signaling pathways result in enhanced ER protein folding potential, increased degradation of unfolded proteins, and decreased protein synthesis. Figure adapted from "UPR Signaling (ATF6, PERK, IRE1)", by BioRender.com (2023). Retrieved from https://app.biorender.com/biorender-templates.

MOTOR BEHAVIOURAL TESTS

Motor behavioural tests are essential for assessing motor capabilities of mice, such as coordination and balance, muscle strength and locomotor activity. Because of the high number of existing tests, a correct selection should be made on four criteria: validity, reliability, sensitivity and utility (Brooks and Dunnett, 2009). Here we present the presumably most representative motor behavioural tests for characterizing the woozy mouse phenotype.

Rotarod test

Rotarod is a simple and common test for a first evaluation of locomotor deficits and coordination of mice. It consists of a circular rod turning at a constant or accelerating speed. Before the test, mice undergo a training trial at low speed, then the real trial is repeated two or three times and latency to fall is recorded (Brooks and Dunnett, 2009). The rotarod test can be performed multiple times at different stages of disease. As drawbacks, animals' weight could affect the performance, it is difficult to distinguish between fatigue and coordination deficits and some animals refuse to stay on the rotating rod.

Grande et al. tested woozy mice on rotarod from 6 to 34 weeks of age, to determine the first manifestations of motor dysfunction and to follow its progression. They found no significative differences from controls until 10 weeks, by which time woozy mice showed a shorter latency to fall up to 16 weeks (Grande et al., 2018). Therefore, the rotarod test is confirmed to be a valid tool to evaluate locomotor deficits but in the case of MSS it is not able to detect alterations in the initial stages of the disease.

Beam walking test

Beam walking assay is useful for assessing fine motor coordination and sense of balance. Mice have to walk across an elevated narrow beam and reach the cage. This test needs two days of training, and then performance is evaluated by measuring the time taken to cross the beam and the occurred number of paw slips (Brooks and Dunnett, 2009; Luong et al., 2011). Beam walking assay is more sensitive than rotarod and does not require expensive equipment, but sometimes mice refuse to walk across the beam or are unable to balance on it. In woozy mice this test is able to reveal subtle deficits in motor functions and balance only at the beginning of the disease, because it is hard to perform with severe ataxic mice (Grande et al., 2018)

Grip strength test

Grip strength test is used to evaluate neuromuscular function by measuring the muscle strength. There are three ways to perform this test: by assessing the ability of the mouse to stick to an inverted wire grid using the four limbs; by measuring the time a mouse can hang on a wire only with its forelimbs; by determining the force needed to pull the mouse off a narrow bar connected to a force transducer (Brooks and Dunnett, 2009). Grip strength test is easy to carry on and effective in monitoring the progression of motor dysfunctions, but its performance could be affected by diet restrictions, handling, body weight and muscle fatigue. Woozy mice show first signs of myopathy at 16 weeks of age (Grande et al., 2018), therefore grip strength assay could be useful to measure the progression of skeletal muscle degeneration in these animals.

CONCLUSIONS

Mouse models are extremely important for the study of disorders affecting movement, such as ataxias, and for the development of effective treatments. A large number of motor behavioural assays has been validated to assess motor capabilities of mice, and the combined use of these tests could provide useful information about MSS. Cognitive tests that are able to evaluate memory, learning process and attention, should be taken into account for a more comprehensive characterization of woozy mouse phenotype.

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