JIM
JIM 2024; 1 (3): 590
DOI: 10.61012_20248_590
Category: *Review*

POTENTIAL APPROACHES FOR THE TREATMENT OF MARINESCO – SJÖGREN SYNDROME

M. VIELE[1,2], A. G. RUGGIERI[1,2], F. BELLIA[1,2], F. POTENZA[1,2], L. AMODEI[1,2], M. SALLESE[1,2]

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[1]Department of Innovative Technologies in Medicine and Dentistry, “G. d’Annunzio” University of Chieti-Pescara, Chieti, Italy
[2]Center for Advanced Studies and Technology (CAST), “G. d’Annunzio” University of Chieti-Pescara, Chieti, Italy

Corresponding Author
Michele Sallese, PhD; e-mail: michele.sallese@unich.it

ABSTRACT – Marinesco-Sjögren syndrome (MSS) is a rare autosomal-recessive genetic disorder for which there is no cure. Patients present with ataxia, muscle weakness, and cataracts, although many other symptoms may be present, including mental retardation, short stature, and nystagmus. From a molecular perspective, a mutation in the *SIL1* gene has been shown to cause MSS; however, the disease initiator has not yet been identified in many cases. *SIL1* encodes an endoplasmic reticulum (ER) nucleotide exchange factor required to release adenosine diphosphate and folded proteins from the chaperone-binding immunoglobulin protein. Loss of *SIL1* function impairs ER protein folding, triggering the unfolded protein response (UPR) to restore ER homeostasis. However, protracted UPR is a major factor in Purkinje cell degeneration and myopathy.

Depending on the timeliness of its administration, a treatment for MSS could cure the syndrome or substantially improve muscle strength and halt the progression of ataxia. To date, we have accumulated sufficient information to implement preclinical treatments partly due to the knowledge obtained in animal models representative of the human syndrome. Inhibition of ER PRK-like kinase arms of UPR improved the motor performances of a *woozy* mouse model of MSS, delayed Purkinje degeneration, and reduced skeletal muscle alterations. However, the beneficial effect of this treatment diminished over time and was toxic to the pancreas. Another potential approach was experimented on immortalized lymphoblasts from patients. In this case, the chemical chaperone tauroursodeoxycholic acid reduced apoptosis of lymphoblasts. Taking cues from other recessive diseases, it would also be possible to reintroduce wild-type *SIL1* to restore the missing function, but no such evidence has been reported so far. In conclusion, although not yet available, there is good guidance on approaching the treatment of MSS.

Keywords: Ataxia, Chaperone, Marinesco-Sjögren syndrome, Myopathy, Neurodegenerative diseases, SIL1, Unfolded Protein Response.

List of abbreviations: AAV: adeno-associated virus; ATF4: activating transcription factor 4; ATF6: activating transcription factor 6; BiP: binding immunoglobulin protein; CFTR: cystic fibrosis transmembrane conductance regulator; CHOP: C/EBP homologous protein; eIF2α: eukaryotic translation initiation factor 2A; ER: endoplasmic reticulum; ERAD: ER-associated degradation; FRDA: Friedreich ataxia; FXN: frataxin; HSV: Herpes simplex virus; IQGAP1: IQ motif-containing GTPase activating protein 1; IRE1: inositol-requiring enzyme 1; MSS: Marinesco–Sjögren syndrome; ORP150: oxygen-regulated protein 150; PERK: ER PRK-like kinase; SMN1: survival motor neuron; TUDCA: tauroursodeoxycholic acid; UPR: unfolded protein response; WT: wild-type; XBP1: x-box binding protein 1.

INTRODUCTION

Marinesco–Sjögren syndrome (MSS) is a very rare neurodegenerative diseases at early onset. Its worldwide prevalence is not known precisely; it is thought to be about 1 in 1,000,000[1]. MSS is classified as a genetic disease; in about 60% of cases, it is caused by a mutation in the *SIL1* gene and is inherited in an autosomal recessive manner. Many of pathogenic variants of this gene have been described, but no consistent correlation between genotype and phenotype has been found[2-5]. Indeed, the clinical manifestations are highly variable, and although SIL1 is a ubiquitously expressed protein, only specific tissues, such as the cerebellum, muscles and lens of the eye, are overtly diseased; the explanation for this is still unknown[6]. In line with the above, the main symptoms, also used to make a diagnosis, are cerebellar ataxia, myopathy, and bilateral cataracts (Figure 1)[7]. In the range of clinical variability, we find several other flaws (see below) that contribute to the overall picture but are not always present. The first symptom to appear is cataracts, which in many cases may be congenital; if not, they occur early in life and usually affect both eyes. Cataracts progress rapidly and usually require removal of the opacified lens around the age of 10 years[8]. From the first year of life, at the muscular level, patients exhibit hypotonia with muscle weakness, a condition sometimes referred to as “floppy baby,” myopathy, and delayed psychomotor development. Muscle fibers degenerate, and biopsy analysis often reveals replacement with fibroadipose tissue and altered fiber size, as well as abnormal interfibrillar membranous structures[9,10]. It is not yet clear when exactly the alteration of the cerebellum occurs, but this is due to the degeneration of Purkinje neurons, causing ataxia with balance disturbances and cerebellar atrophy. The combination of both muscle and cerebellum impairments causes children to experience delays in reaching developmental milestones and language skills. Lesions to the cerebellum may cause uncoordinated and synergistic activity of skeletal muscles (dyssynergia), excessive or insufficient movements relative to the intended action (dysmetria), difficulty in performing rapidly alternating movements and in opposite directions (dysdiadochokinesia), and problems with stance and gait.

Motor functions are impaired during growth until a plateau is reached, which varies for each case in both age and severity, but most patients do or will need walking aids[1]. Patients with MSS are usually short in stature, and some of them may have skeletal deformities (Figure 1). Some individuals can also

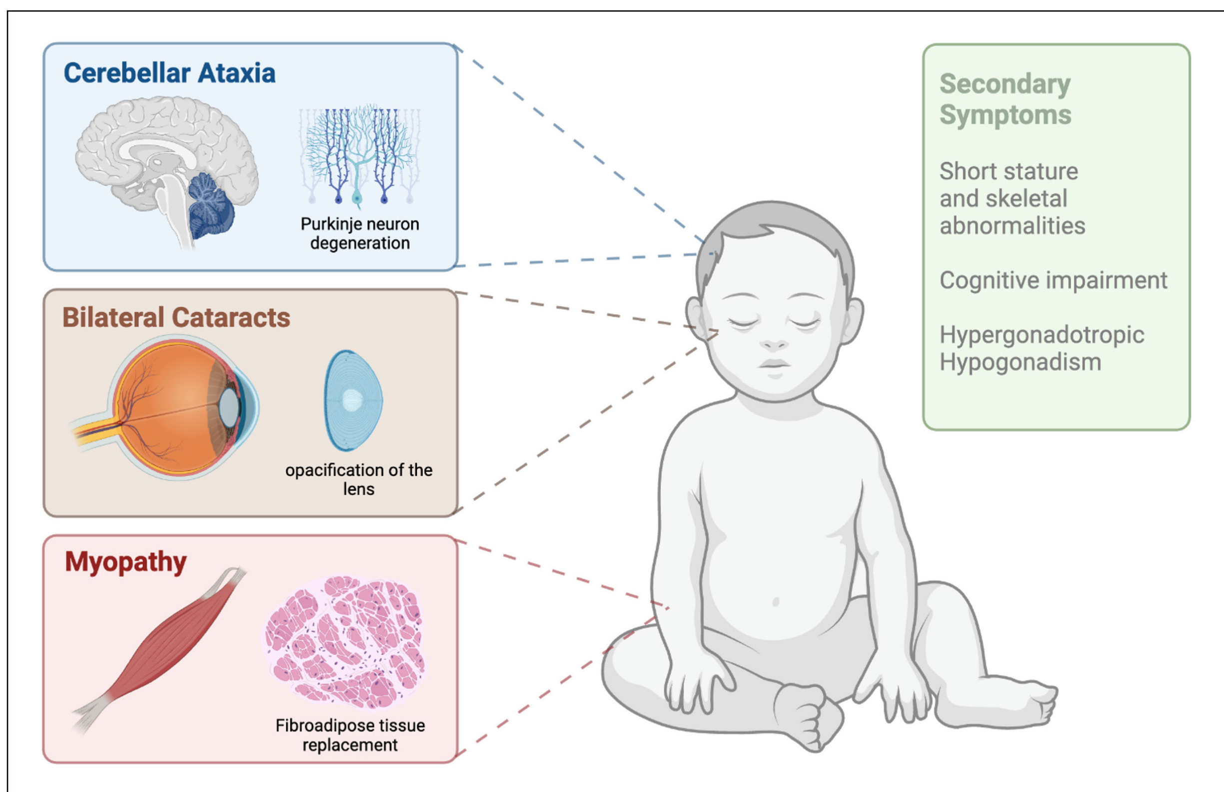

Figure 1. Main clinical signs of patients affected from Marinesco-Sjögren syndrome.

present with mild to severe mental retardation because of the role of SIL1 in neuronal morphology and migration during development[11,12]. Primary gonadal insufficiency is another frequently observed symptom; reduced production of sex hormones contributes to short stature and leads to delayed puberty and the development of secondary sexual characteristics[1].

Although MSS is a fairly disabling multiorgan disease, it does not appear to shorten lifespan.

Diagnosis is based on clinical evaluation of the main symptoms by performing magnetic resonance imaging for cerebellar atrophy, ophthalmologic checks to identify cataracts, and muscle biopsies to reveal myopathy[1]. Genetic tests that can detect biallelic mutations in the *SIL1* gene are used to confirm clinical observations[1].

Currently, there is no cure for this disease. Except for cataracts, which are corrected by surgery, only supportive therapies can be provided to slow all other problems worsening. Hypergonadotropic hypogonadism is treated with hormone therapy that enables patients to prevent osteoporosis. Psychomotor difficulties are usually monitored and followed up by neurologists and physical therapists. The high variability among patients and the rarity of the pathology certainly makes understanding the pathological mechanisms very tortuous. However, by exploiting animal models for preclinical studies in combination with personalized medicine, progress can be made toward a treatment.

MOLECULAR MECHANISMS INVOLVED IN MSS

SIL1 protein contributes to maintaining the endoplasmic reticulum (ER) homeostasis by facilitating protein folding. An impairment in the molecular folding machinery is the main consequence of mutations in the *SIL1* gene in MSS[13].

SIL1 is a nucleotide exchange factor of the binding immunoglobulin protein (BiP), the main chaperone of ER[14]. To carry out its many functions, including protein folding, BiP uses adenosine triphosphate, which, when hydrolyzed, is released with the help of SIL1. Since *SIL1* mutants are often degraded or fail to interact with BiP, there is an overload of misfolded proteins within ER and subsequent activation of the unfolded protein response (UPR)[6]. Reducing protein synthesis, increasing folding capacity, and removing misfolded proteins via ER-associated degradation (ERAD) are the main processes enacted by UPR[15]. These tasks are performed by three ER transmembrane stress sensors: ER PRK-like kinase (PERK), inositol-requiring enzyme 1 (IRE1), and activating transcription factor 6 (ATF6)[15].

PERK phosphorylates the eukaryotic translation initiation factor 2A (eIF2α), suppressing general protein synthesis but allowing translation of specific transcripts, such as activating transcription factor 4 (ATF4). ATF4 triggers the activation of several downstream effectors, such as the pro-apoptotic C/EBP homologous protein (CHOP)[15]. The IRE1 kinase employs its ribonuclease activity to splice the transcription factor x-box binding protein 1 (XBP1) and degrade several mRNAs via regulated IRE1-dependent decay[16]. ATF6 is a transcription factor that is activated through proteolytic cleavage in the Golgi and translocated into the nucleus, activating the transcription of several UPR genes[15]. UPR is a prosurvival response, but it leads to apoptosis when stress is chronic and cannot be overcome. The human genome expresses the oxygen-regulated protein 150 (ORP150) chaperone, which has nucleotide exchange activity toward BiP; thus, it could replace the loss of SIL1 activity. A study showed that overexpression of ORP150 prevents ataxia and muscle problems in *woozy* mice[17]. Unfortunately, spontaneous overexpression of ORP150 is not sufficient to replace the lack of SIL1.

We can speculate that the degree of UPR activation is related to cellular damage. UPR activation is limited in non-clinically diseased cells, such as fibroblasts, whereas it becomes markedly more prominent in skeletal muscle and Purkinje cells, as observed in preclinical models[10,18-20].

In agreement with this hypothesis, in our previous proteomic study, we found that MSS fibroblasts cope with SIL1 deficiency through mild activation of UPR and strong downregulation of the ribosomal machinery along with metabolic alterations to reduce ER protein overload[18].

REPRESENTATIVE ANIMAL MODELS OF MSS

Two different mouse models carrying the *SIL1* gene mutation have been identified/generated in the last few years. The first one, known as the *woozy* mouse, is characterized by a spontaneous recessive mutation, while the $SIL1^{GT}$ mouse is a genetically engineered model[19]. Furthermore, zebrafish models with *SIL1* knockdown have been developed[21,22].

In 2005, Zhao et al[19] described a mouse, called a *woozy* mouse, that expresses a truncated SIL1 protein resulting from inserting an early transposon element next to exon 7 on chromosome 18 and exhibits the typical clinical signs of MSS[19]. In this original study, the authors showed that cerebellar ataxia appears in these animals around 12–16 weeks of life because of Purkinje cell degeneration from cerebellar lobules I–VIII[19]. Through calbindin immunostaining, Grande et al[23] pointed out that these neurons begin to degenerate as early as 8 weeks of life[23]. UPR activation is detectable from week 6 onward, with increased phosphorylation levels of eIF2α and increased mRNAs of the pro-apoptotic factor CHOP and transcription factor *XBP1*, which activate genes involved in protein folding, secretion, and ERAD[23]. Signs of apoptosis in Purkinje neurons were revealed by immunofluorescence analysis, with activation of caspase-3 and the presence of nuclear double-strand breaks in DNA evidenced by the TUNEL assay. Moreover, electron microscopy highlighted condensed nuclear chromatin and autophagosome-like structures[9].

From a functional standpoint, *woozy* mice have progressive degenerative myopathy, comparable to the human MSS phenotype[6,10]. In particular, muscle biopsy revealed morphological changes in the sarcoplasmic reticulum, autophagic vacuoles, swollen mitochondria, and alterations in myonuclei. UPR is also activated in the muscles of *woozy* mice, and misfolded proteins are degraded through the ERAD complex[10].

These molecular alterations manifest phenotypically from 10 weeks of age, when the first signs of motor dysfunction are detectable, as shown by the results of the accelerated rotarod test performed on groups of wild-type (WT) and *woozy* mice from 6 to 34 weeks of age[23]. Therefore, because of its similarities with the human form of MSS, the *woozy* mouse is a relevant model for better understanding its pathological mechanisms and developing potential therapies.

Another murine model of MSS is the *SIL1^{Gt}* mice, generated by a gene-trapped method on C57BL/6J strain[19]. The mutated SIL1 transcript is truncated after exon 7, resulting in a phenotype very similar to that of the *woozy* mouse, thus confirming the implication of SIL1 in skeletal muscle physiology and neurodegeneration[20]. In 2018, Ichhaporia et al[20] evaluated the muscle strength of these animals by measuring their ability to cling to an inverted screen, compared with WT mice[19,20]. Results indicated that around the 24th week of life, there was a progressive decrease in muscle strength, supported by structural abnormalities in muscular tissue, such as irregular fibers and myofibrillar disorganization with adipose infiltration, which were revealed by histological and electron microscopy analysis[20]. Furthermore, a proteomic analysis of *SIL1^{Gt}* mice quadriceps showed significant alterations in the expression of 515 proteins compared with WT muscles. These changes involved a variety of cellular compartments and important physiological pathways, such as cation channel activity, insulin receptor signaling, and glucose metabolism. However, increased glucose uptake in the skeletal muscle of SIL1^{Gt} mice appears to be caused by altered insulin and insulin-like growth factor 1 signaling and increased glucose transporter 4 (GLUT4), suggesting partial metabolic compensation in response to the loss of muscle mass and strength[20]. *SIL1*^{Gt} mice do not display bilateral cataracts like MSS patients, but sporadically unilateral cataracts occur[7].

Concerning neurodegeneration, *SIL1* gene mutation has also been associated with the development of cerebral cortex[12]. Indeed, when SIL1 was silenced in mouse cortical neurons, they showed a delay in corticogenesis, evidenced by an altered distribution of these cells among the different layers of the cerebral cortex[12]. Along these lines, a proteomic analysis conducted on mouse cortical neurons in which the *SIL1* gene was silenced by shRNA revealed changes in the expression of proteins related to actin cytoskeleton[11]. Western blotting confirmed the decreased expression of actinin-1 and vimentin, while IQ motif-containing GTPase activating protein 1 (IQGAP1) was increased. These results suggest that SIL1 may play a role in actin dynamics, which in turn is involved in neuronal migration and probably affects the cognitive impairment reported in MSS[11].

To further understand the primary characteristics of MSS, antisense oligo morpholino was directly injected into the eggs of zebrafish (Danio rerio) to silence the expression of the *SIL1* gene[21,22]. Reduced expression of SIL1 protein in these animals generated a phenotype reminiscent of MSS. In particular, birefringence assay, in which the fish is placed between two polarized filters, revealed altered structural organization of muscle fibers[21]. Muscle abnormalities, such as altered myofiber formation and V-shaped structure, as well as alterations in neuromuscular junctions, were confirmed by immunostaining[21,22]. In addition, the *SIL1$^{-/-}$* zebrafish exhibited fewer Purkinje cells and smaller eyes than control[21].

PHARMACOLOGICAL TARGETING OF UPR AS A PROSPECTIVE TREATMENT OF MSS

MSS belongs to a large group of misfolding diseases causing neurodegenerative disorders such as Alzheimer's, Parkinson's, amyotrophic lateral sclerosis and prion disease[24-27]. Although these disorders are caused by different gene mutations and have completely dissimilar etiological and pathological mechanisms, they all show activation of UPR[24-27]. All three UPR sensors can be activated in misfolding diseases, but PERK is probably the most pathologically relevant. As reported above, PERK activation by phosphorylating eIF2α inhibits the general protein synthesis[28]. This heavily affects proteostasis, leading to the deficiency of important proteins for neuronal survival, including the synaptosome-associated protein 25, postsynaptic density protein 95, and N-Methyl-D-Aspartate receptor NR1 subunit[27]. Therefore, dysfunctional UPR can contribute to neurodegeneration by depleting key synaptic proteins. Intending to restore protein synthesis and, consequently, synaptic function, some studies have targeted PERK with promising results[29].

Along these lines, we generated a cellular model of MSS (SIL1 knockdown in HELA cells) and tested the effect of the PERK inhibitor GSK2606414[30]. Interestingly, cells treated with GSK2606414 had reduced apoptosis, increased proliferation, and a more efficient secretory pathway[30]. Building on this original finding and literature data, we examined improvements in the motor function of *woozy* mice treated with GSK2606414 (Figure 2). Treatments were administered orally at a presymptomatic stage (4 weeks of age) when motor function, UPR activation, and Purkinje cell morphology were comparable to those of heterozygous control mice[23]. Motor performances were monitored weekly using the beam walking test and the accelerating rotarod. *Woozy* mice treated with GSK2606414 performed similarly to healthy heterozygous animals on the beam walking test until 8 weeks of age. At longer times, ataxia, as measured on the rotarod[31], worsened the animals' performance, which nonetheless remained significantly better than in untreated mice (11 weeks of age); thereafter, the benefits of treatment declined further, although they were still detectable at 16 weeks of age[23]. The decline in motor performance was parallel to the reduction in Purkinje dendrite arborization. In fact, the amount of Purkinje dendrites in the treated *woozy* mice was similar to that of healthy controls up to 9 weeks of age, while it was much lower at 16 weeks of age, although still higher than that of untreated *woozy* mice[23]. The benefit of PERK inhibition was also evident in skeletal muscle, as ultrastructural abnormalities, such as sarcoplasmic reticulum enlargement and autophagic vacuoles, were reduced with treatment[23].

The success of the treatment is probably due to the upregulation of ORP150, which can replace the nucleotide exchange activity of SIL1 on BiP, as well as the reactivation of protein synthesis[23]. Conversely, the loss of efficacy over time could be caused by the accumulation of huge amounts of misfolded protein in ER due to active protein synthesis in a system with reduced folding capacity. Another shortcoming of this approach was the pancreatic toxicity of the PERK inhibitor, probably because UPR is part of a physiological response involved in insulin production, especially in the postprandial period.

The multiple drawbacks of PERK pathway inhibition for treating MSS should not be discouraged because most of them can potentially be overcome. For example, we can manage pancreatic toxicity using drugs that directly control eIF2α[32]. Meanwhile, the use of suboptimal concentrations of inhibitors might allow partial recovery of protein synthesis without overloading the inefficient ER folding machinery[32]. A recent study showed that PERK inhibitors, when reaching micromolar concentrations, can lead to hyperactivation of the general control non-depressible (GCN) kinase. GCN is part of the integrated stress response, and phosphorylating eIF2α inhibits protein synthesis[33]. These new data might suggest that the effect of GSK2606414 treatment in woozy mice should be primarily attributed to the upregulation of ORP150 rather than the reactivation of protein synthesis.

Finally, the effect of inhibiting the other arms of UPR (IRE1 and ATF6) on the treatment of MSS has not been studied, although we cannot exclude that their modulation may impact the progression of MSS.

CHEMICAL CHAPERONES AS A POTENTIAL TREATMENT FOR MSS

The accumulation of one or more misfolded proteins that damage cells and the quality control mechanisms that recognize aberrant conformations and shunt these proteins toward degradation rather than their final destination contribute to the pathological mechanisms of misfolding diseases. This scenario can lead to either a gain of function (protein aggregates) or a loss of function because the protein is no longer available. The most obvious approach to overcoming this problem is to restore the correct conformation

of the altered protein(s). Indeed, several preclinical studies demonstrated that small compounds could bind to the misfolded protein via electrostatic or hydrophobic bonds and shift the equilibrium toward the correct conformations[34,35]. Some of these chemical chaperones have been approved for the treatment of human disease. For example, Fabry disease is caused by mutations in the lysosomal enzyme alpha-galactosidase A involved in glycolipid metabolism[36]. Treatment with migalastat favors the correct folding of the enzyme and, thus, the recovery of the function unless the mutation affects the active site[37]. In transthyretin amyloidosis, a disease caused by the accumulation/precipitation of transthyretin in different tissues, the treatment with tafamidis was able to stabilize the quaternary structure of transthyretin and thus reduce the formation of clumps[38,39]. Cystic fibrosis is caused by mutations in the chloride channel of the cystic fibrosis transmembrane conductance regulator (CFTR). To date, pharmacological chaperones, such as ivacaftor have been approved to improve chloride ion passage by promoting channel opening. Interestingly, the combination of pharmacological chaperones, including ivacaftor, tezacaftor, and elexacaftor, has also been approved to promote CFTR folding, transport to the plasma membrane, and opening[40,41]. Note that these pharmacological chaperones are not effective for any *CFTR* mutation but only for specific mutations. These few examples underline the potential of pharmacological chaperones.

The effects of chemical chaperones were tested with the aim of rescuing the MSS phenotype in cultured cells. Specifically, immortalized lymphoblastoid cell lines were generated from four patients carrying the characteristic p.Leu313fs *SIL1* mutation widely prevalent in Japanese patients[3,42]. These cells showed UPR activation, increased cell death, and high sensitivity to treatment with tunicamycin (a UPR-activating glycosylation inhibitor) compared with lymphoblastoid cell lines from control individuals[42]. The initial screening included valproate, dexamethasone, 4-phenylbutyric acid, and tauroursodeoxycholic acid (TUDCA), chemical chaperones previously shown to relieve ER stress and protect against tissue damage[42].

TUDCA was able to significantly reduce tunicamycin-induced cell death in patient-derived lymphoblastoid cells, while the other compounds were ineffective[42]. However, treatment with TUDCA (**Figure 2**) was not effective in reducing the levels of the stress markers ER, BiP, and the splice of XBP1[42]. Mechanism of action analysis indicated that TUDCA reduces both mitochondrial depolarization as well as activation of caspases and poly (adenosine diphosphate-ribose) polymerases[42]. Nothing is known about the effect of TUDCA on the folding, stability, or functions of SIL1. These experiments suggest that chemical chaperons are potentially useful in treating MSS. However, as reported for other misfolding diseases, the chemical chaperone should be tailored to the target protein and the specific mutation. Drug screening could lead to the discovery of small molecules interacting with SIL1, possibly affecting its folding and functions. Of course, not all mutations are amenable to this approach.

In conclusion, we believe that in the future, the identification of SIL1-specific chemical chaperones, possibly used in combination with proteostasis regulators, such as PERK pathway inhibitors or proteasome inhibitors, may contribute to the effective treatment of MSS (Figure 2).

PROSPECTIVE GENE THERAPY FOR THE TREATMENT OF MSS

Regarding MSS, gene therapy presents an exciting opportunity to correct the underlying genetic problems. Specifically, the focus is on mutations in the *SIL1* gene[43,44], which are present in 60% of MSS cases [2]. Using the rapid advancements in gene therapy and the emergence of next-generation viral vectors, there is the possibility of delivering long-term benefits with a single dose[45]. These viral vectors have shown an excellent safety profile, a wide tissue distribution capacity, and clinical efficacy in several types of diseases[46,47].

Similar in some phenotypical/clinical features to those reported by MSS patients, Friedreich ataxia (FRDA) is the most common autosomal recessive cerebellar ataxia, caused by a GAA triplet expansion in the frataxin (*FXN*) gene[48]. FRDA patients show a deficiency of frataxin protein in mitochondria. Physiologically, FXN is mainly expressed in tissues with a high metabolic rate[49]. Through conditional silencing of the FXN in cardiac tissue and cerebellum, mouse models with clinical features similar to FRDA were developed[50]. These models are very useful for testing the potential of different treatments for FRDA. In these preclinical models, treatment with Herpes simplex virus (HSV)-BDNF resulted in a decrease in apoptosis and an increase in calbindin levels in the cerebellum of mice, together with an improvement in motor coordination[50]. In a loxP(FRDA) mouse model created by Lim et al[51], FXN was selectively depleted in the inferior olive of the cerebellum, causing motor deficits. They observed an improvement in motor capacities in the accelerating rotarod test using an HSV-1 vector delivering human frataxin, which is functional evidence of the restored protein levels verified by western blotting[51].

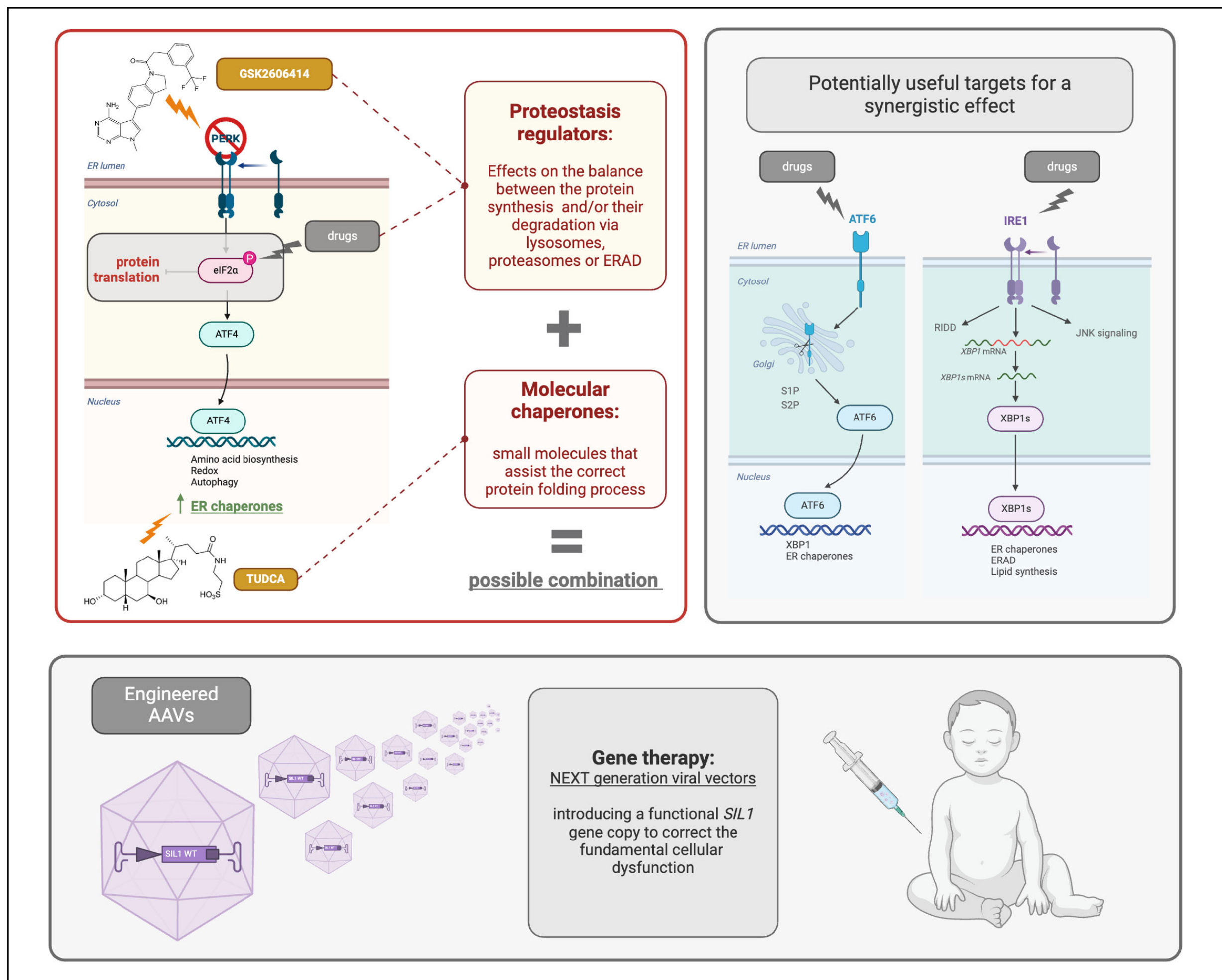

Figure 2. Potential treatments for Marinesco-Sjögren syndrome.

The adeno-associated virus (AAV) vector is a similar approach to the HSV-1 vector, delivering genetic material into the cells. In fact, improvements in cardiac functions have been reported in other mouse models of FRDA using AAV9 delivering human frataxin[52]. Investigators detected improvements in the treated mice's cardiac function and overall life survival, emphasizing the significance of such therapy for FRDA. Salami et al[53] adopted a similar strategy, delivering FXN in a mouse model where the protein is precisely depleted in the heart. After receiving AAVrh10-FXN therapy, the mice's functional cardiac performance was recovered, and they showed similar performance on a treadmill as their littermate controls[53]. Piguet et al[54] used a conditional mouse model with frataxin deletion in parvalbumin-positive cells. This model resulted in sensory ataxia and neuropathy associated with FRDA, showing tremors, progressive movement dysfunction, and loss of coordination. Mice that received AAV9-FXN and AAVrh10-FXN intravenously and intracerebrally, respectively, showed significant improvement in motor and muscle abilities after 1 week[54].

Dual AAV vector technology enables the recombination of vector genomes to express large therapeutic genes, addressing the challenge of the limited transport capacity of standard AAV vectors. This gene therapy is designed to restore dystrophin expression in the muscles of dogs with Duchenne muscular dystrophy, which is comparable to MSS in terms of the mechanisms affecting muscular districts. Significant improvements in biochemistry, histology, and physiology were achieved with this strategy, along with improved muscle protection and strength[55]. Although the *SIL1* gene encodes a 461 amino-acid protein, eliminating the need for a dual AAV vector, this study suggests that gene therapy is in steady development and, with further investigation, could effectively treat a wider variety of neurological diseases.

Clinical trials have also tested and led to the approval of gene reintroduction strategies. For the treatment of spinal muscular atrophy, the US Food and Drug Administration approved onasemnogene abeparvovec in 2019, consisting of AAV9-mediated reintroduction of the survival motor neuron (*SMN1*) gene[56]. The loss of alpha motor neurons, which are crucial for controlling muscle movement, is caused by a deficit of the SMN

protein. This deficiency results in increased symmetric muscle weakening, blocking signals from the spinal cord to the skeletal muscles[57,58]. Patients' prognoses have been proven to improve dramatically with this treatment, which is especially beneficial when administered during the first few months of life[56].

These methodologies could be adapted to MSS by introducing a functional copy of the *SIL1* gene to correct the fundamental cellular dysfunction that causes this syndrome (Figure 2). If successfully implemented, this could alleviate MSS symptoms and thus significantly improve patients' quality of life. Although the clinical features of the disorders reported as examples are different from MSS, and there is no newborn screening for MSS, early treatment with gene therapy in young individuals should be implemented before irreversible damage occurs.

CONCLUSIONS

Several therapeutic approaches have been explored to reduce the deficiencies resulting from SIL1 loss in individuals with MSS. Apart from surgery that can correct bilateral cataracts, other affected tissues pose a challenge to find a similar remedy. However, proteostasis regulators, chemical chaperones, and gene therapy are all potentially useful to varying degrees and with tissue-specific results. The time window of treatment must be carefully considered because of the different timing of disease onset in the cerebellum, skeletal muscles, and eyes. It should be noted that early treatment can arrest Purkinje degeneration, while late treatment cannot recover Purkinje neurons already lost. On the other hand, skeletal muscle can likely be recovered from myoblasts at any time during therapy. These considerations suggest that future treatment might be useful for muscle weakness, but it should be administered as early as possible to improve motor coordination. Unfortunately, today, MSS is often diagnosed after a long wander among medical specialists when it is too late for effective treatment.

Further investigation of the pathology of MSS is needed to identify new predictive biomarkers that would allow its inclusion in the national newborn screening program. It is worth mentioning that the use of multi-omics approaches could play a crucial role in achieving these goals quickly and thus ensure early and effective treatment as soon as it becomes available. Finally, biomarkers will be useful in monitoring disease progression and formulating new therapeutic strategies to halt the onset/progression of MSS.

Artificial intelligence-assisted technologies:

This manuscript was not written with the help of artificial intelligence-assisted technologies.

Authors' contributions:

This study was conceived by Sallese Michele and Viele Marianna, written by Amodei Laura, Bellia Fabio, Potenza Francesca, Ruggieri Anna Giulia, Viele Marianna and edited by Sallese Michele.

Availability of data and material:

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Conflicts of interest:

The authors declare that they have no conflict of interest to disclose.

Consent for publication:

All authors provide consent for the publication of this manuscript.

Ethics approval:

Ethics approval is not required for narrative review.

Funding:

This work was supported by: the NextGenerationEU - MUR, Fondo Promozione e Sviluppo, DM 737/2021 to M.S. Project title: Study of the pathological mechanisms in the Marinesco-Sjögren syndrome by means of a multi-omics approach, acronym: MSS-omics, CUP number: D75F21003210001; The Italian Telethon ONLUS Foundation, Rome, Italy, for the grants N° GGP20092 to M.S. and for the contract to M.V.; The Ministry of University and Research (MUR), Italy, FSE-FESR, under PON RI 2014/2020, I.1-"Innovative PhDs with Industrial Characterization" funded the PhD scholarship (code: no. DOT1353593-1) of Laura Amodei.

Informed consent:

Informed consent is not required for narrative review.

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