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Genotype-phenotype correlation and adaptive proteome reorganization in Marinesco-Sjögren syndrome

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Abstract. Marinesco-Sjögren Syndrome (MSS) causes cerebellar ataxia, myopathy and congenital cataracts in people carrying *SIL1* mutations. SIL1 is an ATP exchange factor for BiP, the major endoplasmic reticulum (ER) chaperone involved in protein folding. SIL1 loss influences BiP activity, leading to ER stress and the activation of unfolded protein response (UPR). Purkinje cells and skeletal muscle fibers are the most sensitive cells to prolonged pathologic UPR, but adverse effects are detectable in other cell types. Currently a clear genotype-phenotype correlation is missing, due to the variable symptomatology and to the discovery of new *SIL1* variants. We decided to focus our attention on two recent works providing different strategies to shed light on the pathophysiology of MSS. In the first one several cellular biomarkers have been evaluated to distinguish between malignant and benign *SIL1* mutations. The other study proposed a proteomic approach to clarify adaptative mechanisms of MSS fibroblasts in response to SIL1 loss. Further investigations are needed to better understand the pathogenesis of MSS and to simplify the diagnosis in patients.

Keywords: fibroblast, Marinesco-Sjögren syndrome, pathogenic mechanisms, SIL1.

INTRODUCTION

Marinesco-Sjögren syndrome (MSS) is described as a rare, early onset, multisystem disorder which is usually characterized by cerebellar ataxia, myopathy, and congenital bilateral cataracts. MSS patients could present additional symptoms including mental retardation, intention tremor, hypergonadotropic hypogonadism, short stature, and skeletal deformities (Anttonen, 2006). Learning disabilities and poor motor coordination are due to cerebellar degeneration, consisting primarily in Purkinje cell loss (Ichhaporia and Hendershot, 2021), while hypotonia occurs because of myopathy associated to gradual substitution of muscle with adipose tissue (Roos et al., 2014). First clear symptoms of the disease manifest after few months from birth, but diagnosis is not so immediate. Regardless, life span appears to be normal (Anttonen, 2006).

In 2005, two different research groups discovered *SIL1* gene mutation in patients affected by MSS (Anttonen et al., 2005; Senderek et al., 2005). In the same year, it was also reported that the spontaneous recessive mutation of *SIL1* (*SIL1*^{wz}) is responsible of Purkinje cells degeneration and myopathy of woozy mice (Zhao et al., 2005). Subsequently, homozygous or compound heterozygous variants of *SIL1* gene have been found in more than 60% of patients. *SIL1* gene encodes for an ATP-exchange factor (SIL1), which is able to bind the endoplasmic reticulum (ER) HSP70 chaperone-binding immunoglobulin protein (BiP). BiP is responsible of protein folding, and SIL1 allows the release of the folded substrate catalyzing the exchange of ADP with a new molecule of ATP (Figure 1). In MSS, mutant *SIL1* generates instable protein that is further degraded, therefore BiP remains associated with the client protein. Consequently, unfolded proteins accumulate in the lumen of ER, inducing the activation of unfolded protein response (UPR) (Chiesa and Sallese, 2020). The UPR is a complex cellular mechanism aimed at reestablishing the normal ER proteostasis by improving ER protein-folding potential, degrading unfolded proteins, and reducing protein synthesis. This is achieved through the activation of three distinct ER transmembrane protein effectors: inositol-requiring enzyme 1 (IRE1), activating transcription factor 6 (ATF6), and protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK) (Restelli et al., 2019). Protracted activation of UPR caused by SIL1 loss induces degeneration of specific cells, namely Purkinje neurons and skeletal muscle fibers. Nevertheless, lack of SIL1 leads to ER stress and functional injuries in other cellular types (Ichhaporia et al., 2015; Ittner et al., 2014; Potenza et al., 2021).

Consistently with the actual knowledge of MSS, a clear genotype-phenotype correlation is missing, because of the variable symptomatology present in some patients, and the increased number of *SIL1* sequence variants, including a heterozygous missense mutation showing cerebellar ataxia without signs of myopathy or cataract

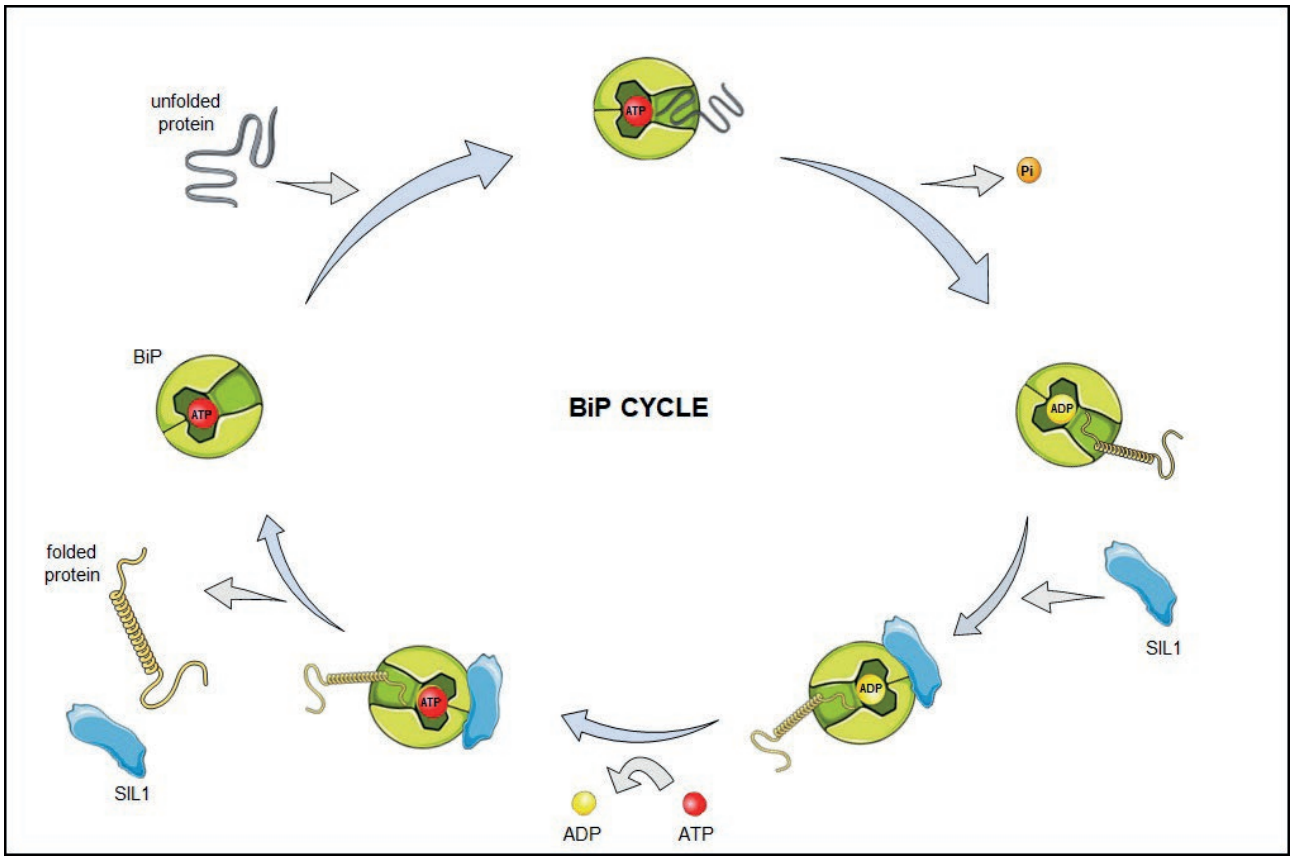

Figure 1. HSP70 chaperone BiP cycle. ATP is needed to bind a new unfolded protein, and its following hydrolysis leads to a conformational change of BiP, which promotes the folding of the client protein. SIL1 protein binds ADP-bound BiP and catalyze the ADP-ATP exchange. Folded protein release occurs in presence of ATP, and then BiP is ready to bind a new unfolded client. When SIL1 protein is missing, BiP remains associated with its client protein, and newly synthesized proteins accumulate in the ER.

(Noreau et al., 2015). In this review we reported two different recent approaches to study cells carrying *SIL1* mutations, with the purpose of clarifying the pathophysiology of MSS.

GENOTYPE-PHENOTYPE CORRELATION ANALYSIS IN MSS

In 2019, Gatz and colleagues examined several cellular biomarkers to evaluate the pathogenicity of *SIL1* mutations in cell models (Gatz et al., 2019). They used Hek293 cells to overexpress 5 different mutant forms of SIL1 protein, including three known malignant mutations (p.V231_I232del, p.G312R, and p.L457P), one missense mutation (p.R92W) with an atypical phenotype (Riazuddin et al., 2009), and one polymorphism as a control (p.K132Q). This work suggested that several read-out measures could be useful to evaluate the pathogenicity of amino acid changes in SIL1 protein. Among them, high molecular weight bands in a native PAGE were signs of aberrant SIL1 protein complexes, along with the presence of SIL1 degradation products. Furthermore, cells with a pathogenic *SIL1* mutation showed reduced metabolic activity and a non-reticular pattern of immunoreactivity. Other distinctive markers were subcellular ultrastructural alterations, such as abnormal mitochondria, vacuoles and protein aggregates, dispersed Golgi, and disintegrated centrosomes (Gatz et al., 2019). In this work SIL1 interactome was also investigated, and this led to the discovery of a disrupted interaction with POC1A (a centriolar protein) and a gain of interaction with DNAJB11/ERj3 (another BiP co-chaperone) by pathogenic *SIL1* mutations. Finally, this study confirmed the increased expression of UPR-/ERAD-related proteins as a common cellular pathogenic biomarker. In conclusion, Gatz and colleagues provided a new approach to distinguish between malignant and benign *SIL1* variants and demonstrated that p.R92W missense mutation could more properly be considered a polymorphism (Gatz et al., 2019).

The importance of defining other read-out measures is becoming more evident for genetic counseling and for characterizing clear genotype-phenotype correlations of MSS patients, especially because of the increasing number of discovered *SIL1* mutations.

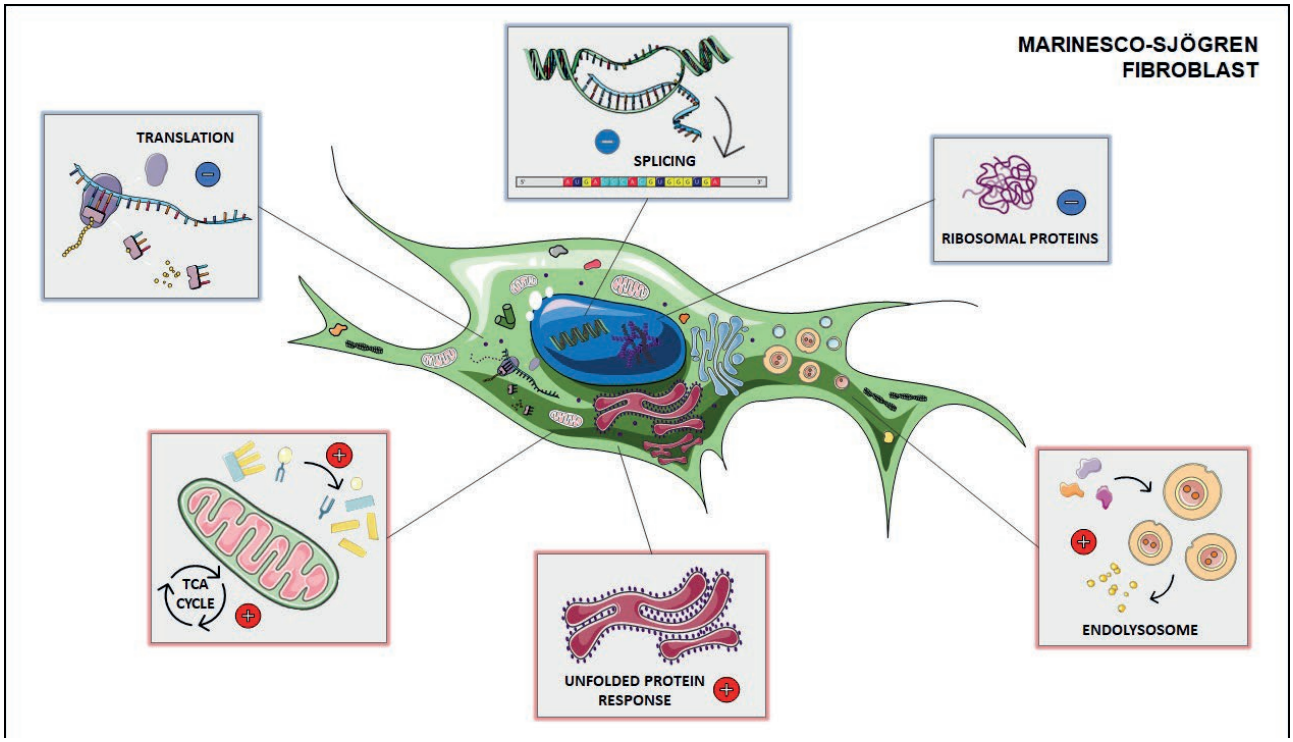

Figure 2. Marinesco-Sjögren fibroblast showing mild signs of UPR. Proteomic analysis revealed downregulation in splicing, ribosome synthesis and translation. TCA cycle is boosted because of enhanced acetyl-CoA production, resulting from increased catabolism of proteins via lysosome-based structure, and reduced biosynthesis of some amino acids. Lipid production is also downregulated, while beta-oxidation is increased.

CELLULAR PROTEOMIC ADAPTATION TO SIL1 LOSS

In 2021 our research group decided to focus on the proteomic adaptation to SIL1 loss in skin fibroblasts collected from a young MSS patient (HF-MSS), affected by *SIL1* R111X mutation (Potenza et al., 2021). Despite fibroblasts are not among the main cellular targets of MSS, mild signs of UPR activation were detected, in addition to an altered cell metabolism. Proteomic analysis on HF-MSS showed differential expression of more than 600 proteins in comparison with control fibroblasts (HF), and after accurate investigations we discovered that these cells reached a senescence-like state to face up to SIL1 loss (Figure 2) (Potenza et al., 2021). In particular, HF-MSS showed an evident downregulation of the spliceosomal complex, as well as ribosomal proteins and RNA translation initiation. Lipids metabolism was also affected with reduced synthesis reactions and increased beta-oxidation. Proteomic analysis also revealed that biochemical pathways leading to acetyl-CoA production were enhanced, resulting in a boosting of TCA cycle and an increased ATP production. ATP, indeed, seems to be more necessary when ER is under stress conditions, and this could explain why MSS fibroblasts undergo metabolic changes when SIL1 protein is mutated (Potenza et al., 2021).

CONCLUSIONS

Marinesco-Sjögren syndrome is not easy to diagnose and there is currently no cure. The detection of new pathogenic cellular biomarkers in addiction to a better understanding of the cell reaction to *SIL1* mutation is useful to bring out genotype-phenotype correlations. Further studies with multi-omics approaches will be needed to learn more about the pathogenesis of Marinesco-Sjögren syndrome.

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