Review of: "Loss of Function of mtHsp70 Chaperone Variants Leads to Mitochondrial Dysfunction in Congenital Sideroblastic Anemia"

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The manuscript written by V Vishwanathan and P D'Silva (Vishwanathan and D'Silva, 2022) is dedicated to the functional characterization of some variants identified in Congenital Sideroblastic Anemia (CSA) in a yeast system.

CSA are rare inherited bone marrow disorders defined by pathological iron accumulation in the mitochondria of erythroid precursors related to abnormal incorporation of iron and mitochondrial dysfunction (Ducamp and Fleming, 2019)(Peoc'h et al., 2019). These are due to defects in heme biosynthesis and/or iron-sulfur cluster synthesis.

In humans, it has been shown that chaperone proteins are implicated in the physiopathology of CSA. For instance, HSPA9 is a mitochondrial chaperone homologous to Hsp70, and biallelic germline pathogenic variants were identified in some CSA patients as an autosomal recessive or pseudo dominant trait (Schmitz-Abe et al., 2015). Another chaperone protein gene has been implicated in non-syndromic CSA, HSCB, the partner of HSPA9. (Ducamp and Fleming, 2019; Crispin et al., 2020)

In this study, a model of yeast was used to incorporate six variants identified in patients with CSA in an mtHsp70 analogous, the chaperon of the yeast (Ssc1) with the particular feature that the yeast has three homologous chaperones. V Vishwanathan and P D'Silva showed different phenotypes in this yeast model: some of the variants (G365S and E392K) were associated with a defect in the thermodependent proliferation related to an abnormal preprotein import across the inner mitochondrial membrane, a decreased functional mitochondrial mass, enhanced ROS concentrations together with increased sensitivity to oxidative stress.

The results presented are clear, convincing, and well presented. Some criticisms may, however, arise about the choice of the model.

First, yeast as a cellular model in a defect in hemoglobin synthesis and anemia sounds surprising. Indeed, yeasts can be both aerobic or anaerobic, and although eukaryotic, they have neither erythrocytes nor circulating hemoglobin.

The second point is that for a single mtHsp70 protein in humans (mtHsp70 or mortalin/Grp75/PBP74), there are three

paralogs in the yeast, according to the authors (Ssc1, Ssq1, and Ecm10). According to others (Sharma and Masison, 2009), humans encode at least eight Hsp70 homologs, and *S. cerevisiae* contains two organelle-specific and six cytosolic Hsp70s. Thus, analyzing the impact of the different variants initially observed in humans in only one of this analogous seems to be somewhat extrapolated. The results would have been more relevant if confirmed in a model with functional heme synthesis as zebrafish, human primary erythroid cells, or mice. Moreover, the reader has some difficulties identifying the localization of the different variants since no sequence alignment between humans and yeast has been provided.

Overall, the results described in the manuscript confirmed the deleterious impact of these loss-of-function mutations. Not surprisingly, mutations in chaperone proteins impacted oxidative stress, ROS levels, mitochondrial mass, and mitochondrial function[KC1] . The role of mitochondrial chaperones proteins in mitochondrial integrity and function and, notably, in mitochondrial DNA integrity is known (Týč et al., 2015). Further investigations should now be undergone to evaluate whether similar defects are observed in cells from patients to confirm the molecular mechanism of heterogenous CSA.

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[KC1]?? que veux tu dire ? ??je proposerai Not surprisingly, mutation in chaperone proteins lead to disruption of oxidative stress and mitochondrial function