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STUDIES IN YEAST MODEL OF PATHOLOGICAL MUTATIONS OF THE HUMAN GENE *HCCS*

QUARTARARO J.*, INDRIERI A.**, FRANCO B.**, FERRERO I.*, GOFFRINI P.*

*) Department of Genetics, Biology of Microrganisms, Antropology and Evolution, University of Parma (Italy)
**) Telethon Institute of Genetics and Medicine and Medical Genetics, Department of Pediatrics, Federico II University of Naples (Italy)

Yeast model, mitochondria, cytochrome c, heme lyase, mitochondrial disease

Microphthalmia with linear skin defects (MLS) is an X-linked dominant male-lethal neuro-developmental disorder associated to mutations in the holocytochrome c-type synthetase (*HCCS*) transcript. Female patients display microphthalmia and linear skin defects, CNS malformation, mental retardation and cardiac defects.

The HCCS gene encodes a mitochondrial protein that catalyzes the attachment of heme to apo-cytochrome c (Cytc) and c1. In yeast the enzyme heme lyase is encoded by nuclear gene CYC3 and then transferred into the mitochondria. Defects in yeast heme lyase (cyc3 null mutant) result in loss development of respiratory growth.

While ectopic expression of human *HCCS* wild-type in a yeast null mutant cyc3 is capable to restore oxidative growth, the expression of *HCCS* mutants associated with MLS disease (E159K; R217C) do not complement the OXPHOS phenotype. Measurement of the mitochondrial cytochrome content were done to evaluate the structural integrity of the respiratory chain complexes. In *cyc3* yeast null strain, transformed with the gene *HCCS* wild type, spectra profile was indistinguishable from the strain carrying the yeast gene *CYC3* wt**.** In contrast, the *HCCS* null strain showed a marked reduction in both the absorption peak of cytochrome c and cytochrome aa3, similar defects were exhibited by the two pathogenic alleles HCCS. In agreement with the reduction in content of cytochrome c we observed a marked reduction in respiratory activity.

Western blotting analysis was performed to check the successful import of cytochrome c into the mitochondria. Pathogenic alleles showed a reduced amount of cytochrome c compared to that of wild-type, indicating the accumulation of apocytochrome c into the mitochondria due to the presence of a heme lyase enzyme, although not catalytically active.

In addition, both strains carrying the *hccs* mutant or null alleles showed a significant decrease in the chronological life span (CLS). Treatment with acetic acid to induce necrosis showed a survival rate of cells of the mutant strains significantly lower than that of wild-type suggesting that mutations in HCCS has led to a decline in life span due to necrotic death. These data confirm the role of *HCCS* in mitochondria and suggest that the MLS should be considered a mitochondrial disease.