Energy deficits and reduced mitochondrial capacity: cause of the extra-skeletal features of hypophosphatasia?

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Introduction

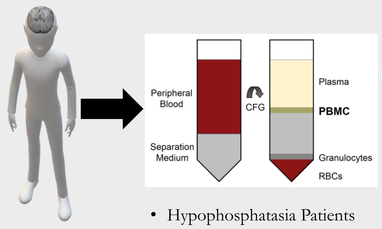
Background: Hypophosphatasia (HPP) is an inherited disorder of skeletal mineralization primarily affecting bones and teeth. HPP is characterized by low circulating levels of tissue non-specific alkaline phosphatase (TNSALP). Mutations in the ALPL gene encoding TNSALP cause HPP. Patients with HPP also have numerous debilitating extra-skeletal manifestations including fatigue, muscle weakness, bone and joint pain, CNS deficits and seizures. Evidence is accumulating that TNSALP is important for regulating mitochondrial energy metabolism.

Objective: To investigate if mitochondrial energy deficits may be the root cause of the extra-skeletal features in HPP.

Methods: We tested this hypothesis in iPSC-lines from 3 patients with childhood-onset HPP due to compound heterozygous ALPL gene mutations (c.571G>A [p.Glu191Lys] and c.1001G>A [p.Gly334Asp]). Peripheral blood mononuclear cells were reprogrammed into induced pluripotent stem cells (iPSCs). These iPSCs were evaluated for pluripotency, trilineage differentiation and chromosomal abnormalities. Mitochondrial function was assessed by comparing cellular lactate dehydrogenase (LDH) and ATP levels along with mitochondrial respiration using Seahorse between the patient and control iPSC lines.

Results

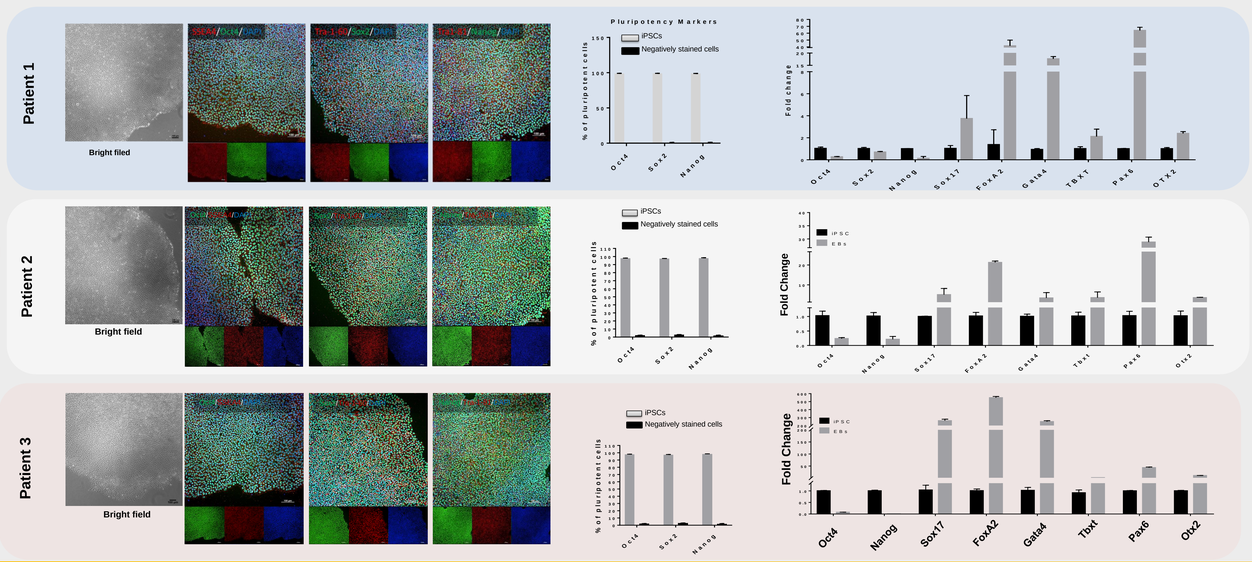
Hypophosphatasia Patients (N=3)



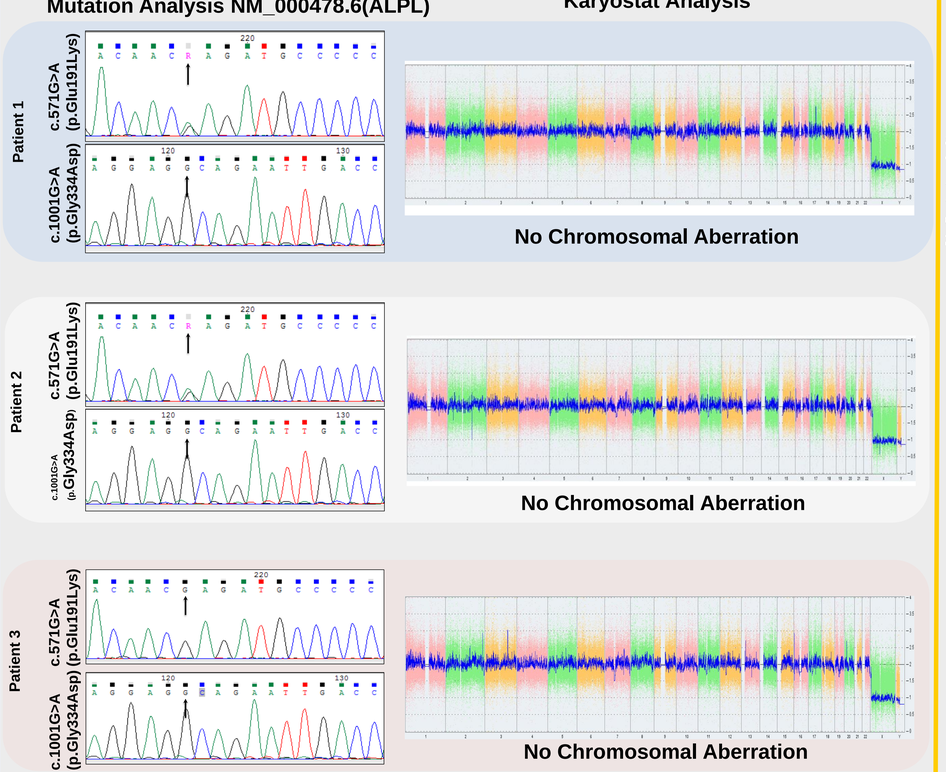
- Hypophosphatasia Patients
- Normal Control

iPSC Reprogramming

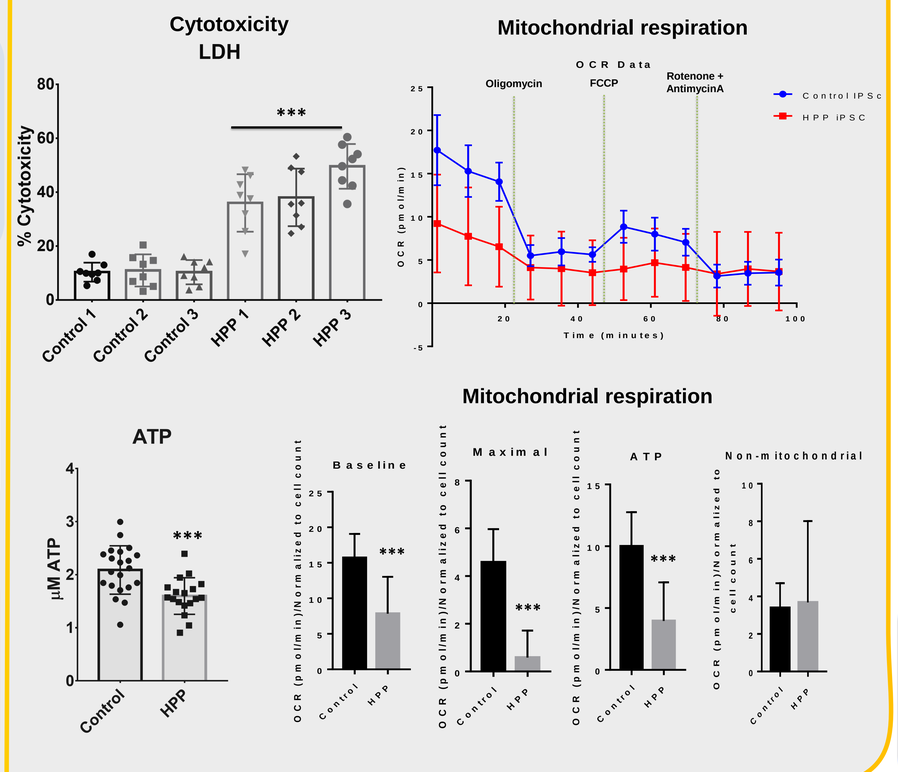
iPSC Characterization



Genetic Screening



Mitochondrial Functional Analysis



Conclusion

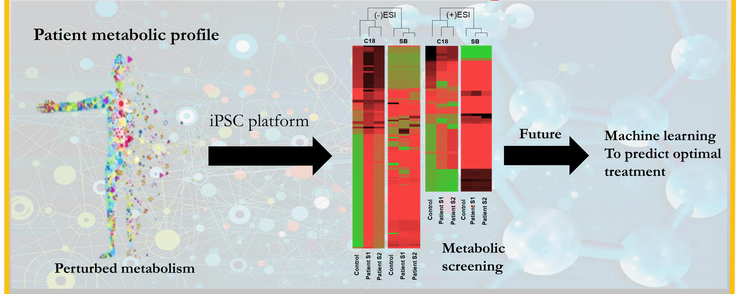
We successfully established HPP patient-specific iPSC cell line models and report the first demonstration of energy deficits and reduced mitochondrial capacity in iPSCs derived from patients with HPP. Mitochondrial dysfunction could be the basis for the extra-skeletal phenotypes of HPP and is a promising target for potential new adjuvant interventions in addition to recently approved enzyme replacement therapy.

Significance

The current study demonstrates an iPSC based platform that can help overcome the genetic variability conundrum and help in generating patient and mutation specific cells to not only study disease the molecular and metabolic mechanisms involved in disease pathophysiology but also help in screening patient targeted drugs to understand their efficacy in respective patients. In the long run this platform could provide improved clinical therapies for patients with Hypophosphatasia.

Future Prospects

Functional assessment of TNALP in patient metabolism will lead to new therapeutic avenues



TNALP influences patient metabolism. If we can map perturbed metabolic pathways, a digital library can be created to predict optimal treatments for future.

Acknowledgements

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