Strategy to compensate the ABCC6 transporter deficiency in PseudoXanthoma Elasticum inherited disease

Laëtitia Clotaire[1,2,3], Georges Leftheriotis[1,3], Saïd Bendahhou[1], Isabelle Rubera [1], Christophe Duranton [1]

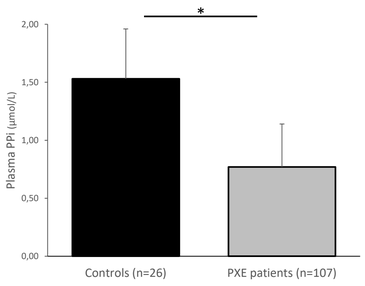
[1] Université Côte d'Azur, CNRS, LP2M, LabEx ICST, 06107 Nice, France. [2] ProNutri, private company, 5ème avenue, 17ème Rue, 06516 Carros, France. [3] Centre Hospitalier Universitaire de Nice, 06000 Nice, France.

Aim of the study

Pseudoxanthoma elasticum
(PXE, OMIM 26 4800)

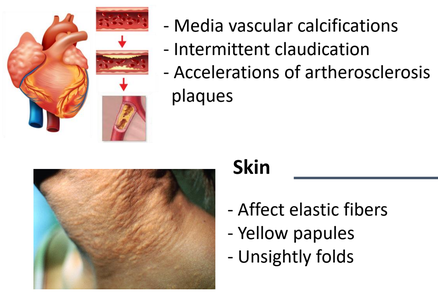
- A rare inherited disease
- Ectopic calcifying disease
- Affecting skin, eyes, arteries, and kidney

- Calcification in PXE results from low plasma level of pyrophosphate (PPi), an anticalcifying molecule.



(Leftheriotis et al., 2022)

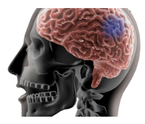
Eyes
- Angioid streaks
- Neo-vascularisation



Cardio-vascular systems
- Media vascular calcifications
- Intermittent claudication
- Accelerations of artherosclerosis plaques

Skin
- Affect elastic fibers
- Yellow papules
- Unsightly folds

Brain
- Ischemic strokes

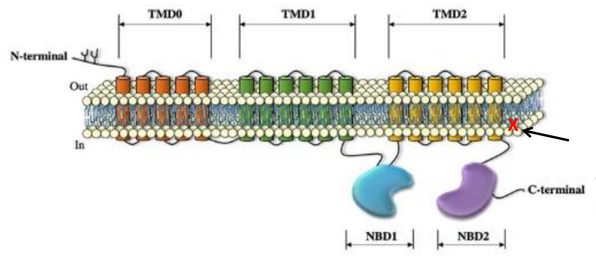


Cardiovascular
- Arterial hypertension
- Coronary/ peripheral arterial disease

Digestive system
- Gastrointestinal bleeding
- Digestive haemorrhages

- Mutation of *ABCC6* gene encoding an ATP-binding cassette (ABC) transporter

Over 300 mutations of *ABCC6* gene
- missense (55%)
- nonsense (15%)
- small deletions (15%)



R1141X
30% of European populations

Exact subtract of ABCC6 protein remains unknown

Results

Therapeutic strategy for PXE patients

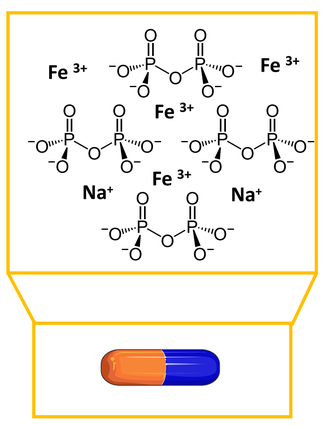
PROPHECI clinical trial (CT) named (NCT04868578)

Phase II randomized controlled CT

Aim:

- **PRIMARY ENDPOINT**
  - Evaluate the efficacy of a daily and oral administration of PPi salts on calcifications
- **SECONDARY ENDPOINT**
  - Evaluate the tolerance and safety of PPi supplementation
  - Obtain PK/PD profil of PPi

PXE patients



Inclusion step (n = 99)
- **Biocollection:** $[PPi]_{pl}$ and $[PPi]_u$ samples
- **Analysis:** Ocular clinical worsening / CT-scan imaging / Dermoscopy /SF36

Randomization step

A Group: **66 Patients**
PPi supplementation
(40mg/kg /day, for 12 months)

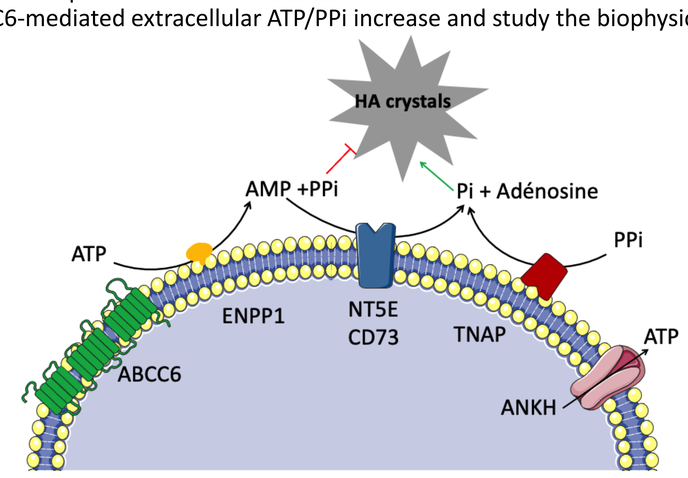
B Group: **33 Patients**
Placebo product
(40mg/kg /day, for 12 months)

$[PPi]_{pl}$ analysis at T_{0min}, T_{30min} and T_{240min}
➢ PK/PD of PPi data

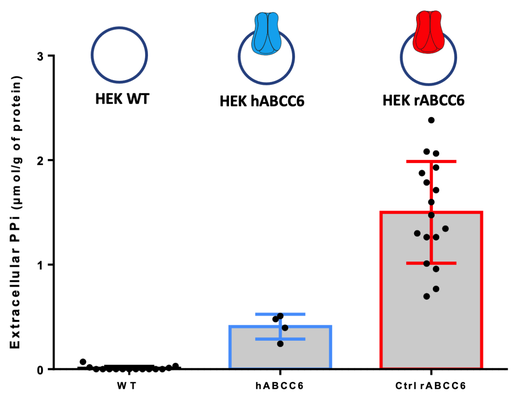
At 12 months, end of study
- **Biocollection:** $[PPi]_{pl}$ and $[PPi]_u$ samples
- **Analysis:** Ocular clinical worsening / CT-scan imaging / Dermoscopy /SF36

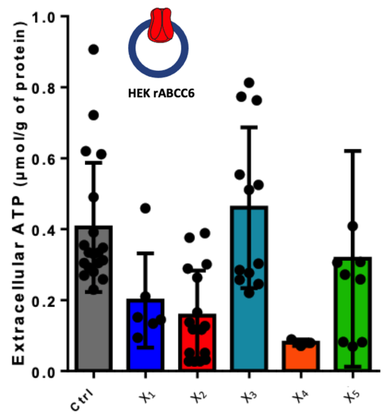
Fundamental and pharmacological mechanisms involving ABCC6 protein

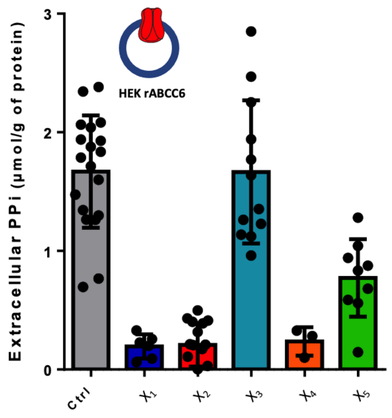
Aim: Explore the role of ABCC6 transporter in the modulation of nucleotides release to identify molecules/pathways which can influence the activity of the ABCC6-mediated extracellular ATP/PPi increase and study the biophysical properties of the transporter.



- **Pharmacological characterization of ABCC6-mediated extracellular ATP/PPi increase on cellular model**



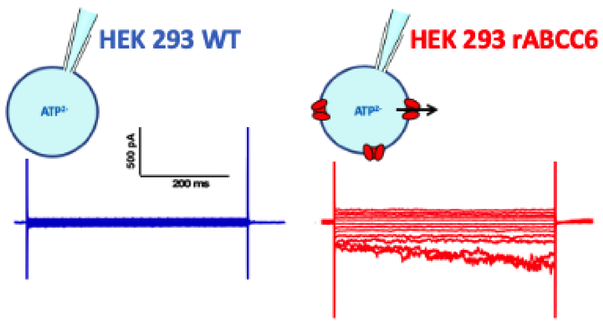


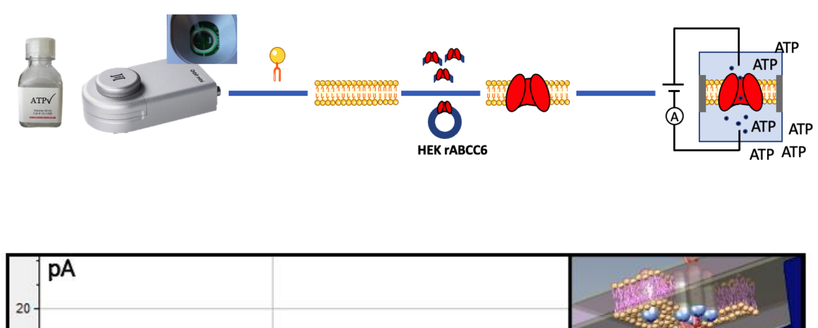


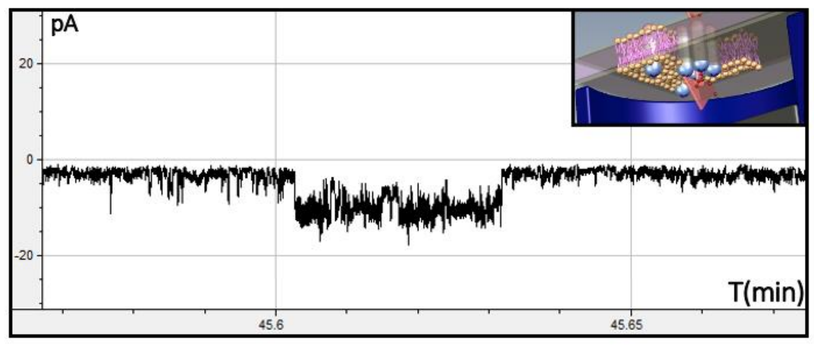
- **Biophysical characterization by electrophysiologic technics**

➢ Recording ABCC6-dependant electrogenic currents by patch clamp whole-cell approach

➢ Monitoring the single channel ATP/PPi-evoked currents using miniaturized horizontal planar lipid bilayer system (Orbitmini, Nanion)







Preliminary data obtained with protein extracted from HEK 293 overexpressing rABCC6

Conclusions

- Identification of 3 potent inhibitors ($X_{1\text{-}2\text{-}4}$) of ABCC6-mediated extracellular ATP/PPi level
- We have recorded for the first time ABCC6-dependent ATP^{2-} currents

These encouraging experiments suggest that ABCC6 could directly or indirectly contribute to the electrogenic transport of ATP/PPi

Perspective: Purification of hABCC6 protein to explore the biophysical properties of ABCC6 transporter to develop innovative treatments

Reference

Leftheriotis, G., Navasiolava, N., Clotaire, L., Duranton, C., Le Saux, O., Bendahhou, S., Laurain, A., Rubera, I., Martin, L., 2022. Relationships between Plasma Pyrophosphate, Vascular Calcification and Clinical Severity in Patients Affected by Pseudoxanthoma Elasticum. JCM 11, 2588.