4-phenylbutyrate therapy restores the calcification inhibition potential of *ABCC6* missense mutations

Hi'ilani Hopkins

ABCC6

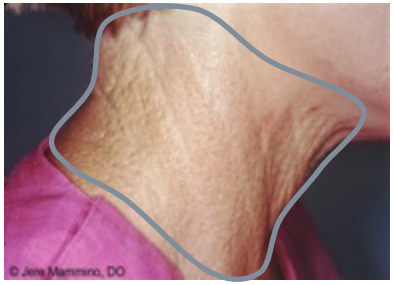
- Active (ATP-dependent) transporter
- Helps to inhibit calcification through pyrophosphate production
- Expressed in the liver, kidney, and intestine
- Localized in the plasma membrane
- Mutations lead to genetic diseases (PXE and GACI)

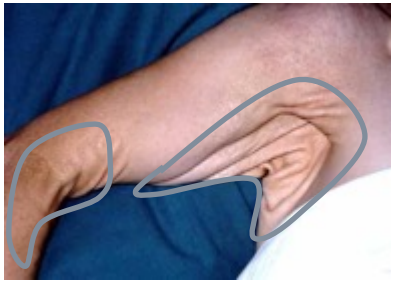
Bergen et al., 2000; Le Saux et al., 2000; Ringpfeil et al, 2000

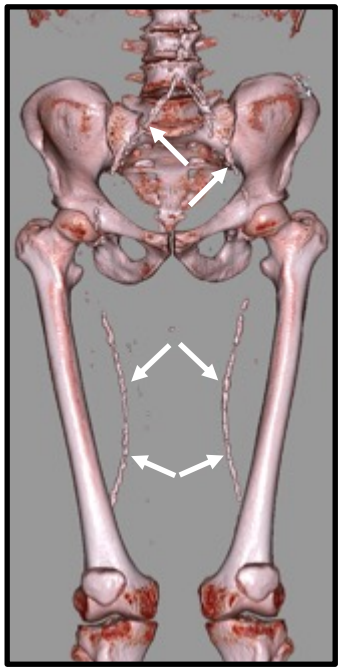
Pseudoxanthoma Elasticum (PXE)

Human Phenotype

Dermal

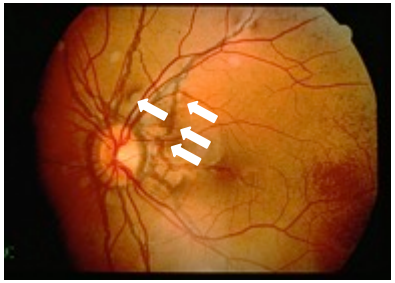


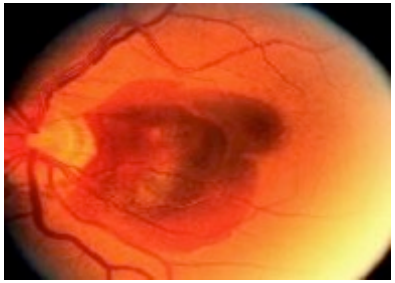




Vascular

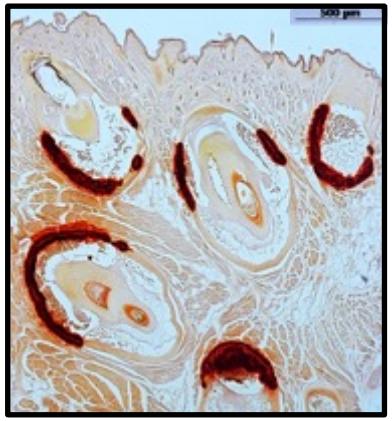
Ocular



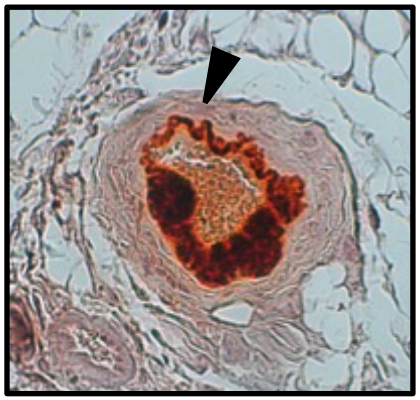


Mouse Phenotype

Abcc6$^{-/-}$ Whiskers



Abcc6$^{-/-}$ Artery



Abcc6$^{-/-}$ eyes (Bruch's membrane)

Hypothesis

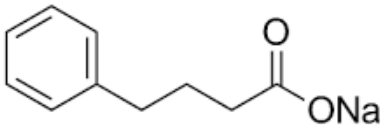
- 4-phenylbutyrate (4-PBA)
  - drug used to treat urea cycle disorders
  - also interferes with ER chaperones

- Most ABCC6 mutants have normal transport activity, but abnormal cellular localization, which can be rescued by 4-PBA (Le Saux et al., 2011; Pomozi et al., 2014)

- **Because 4-PBA rescues plasma membrane localization of several ABCC6 mutants, we hypothesize that it will also rescue ABCC6's function *i.e.* calcification inhibition**

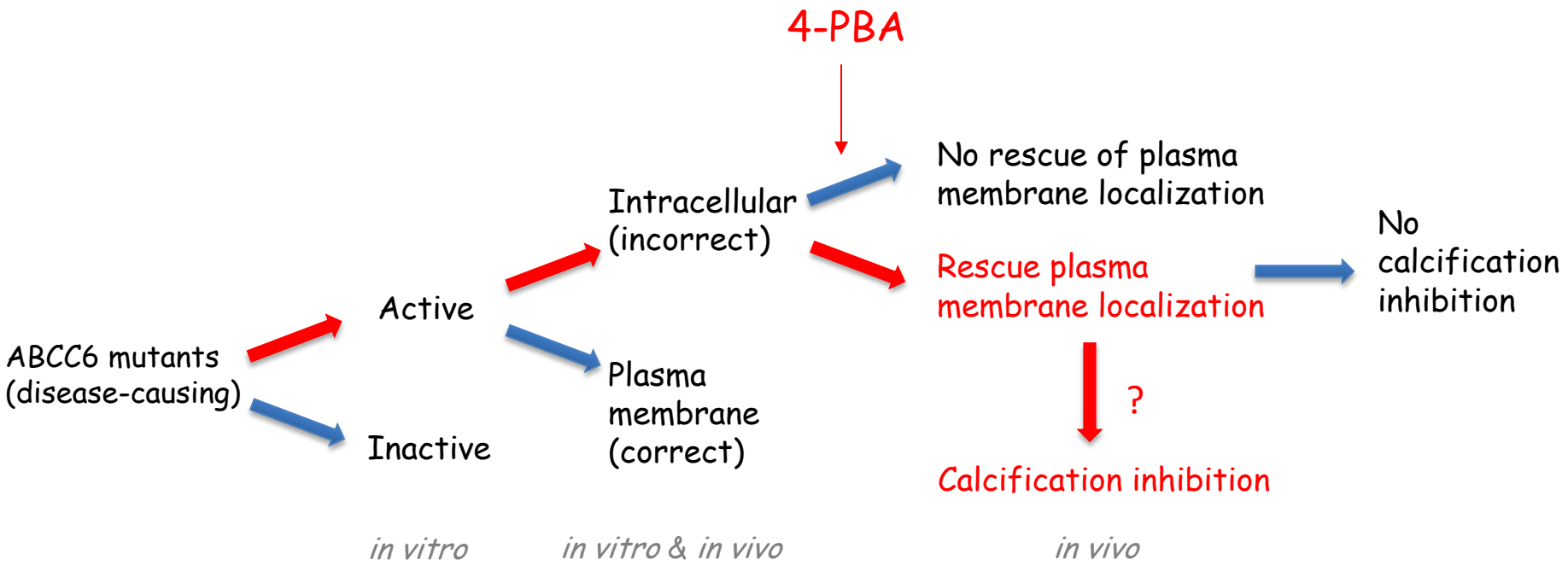
4-PBA interferes with ER chaperones

- Inducing Hsp-70 and Hsp-90
- Inhibiting Hsc-70 and CHIP



Purpose

Can 4-PBA restore plasma membrane localization of various ABCC6 mutants and stop/prevent further calcification?

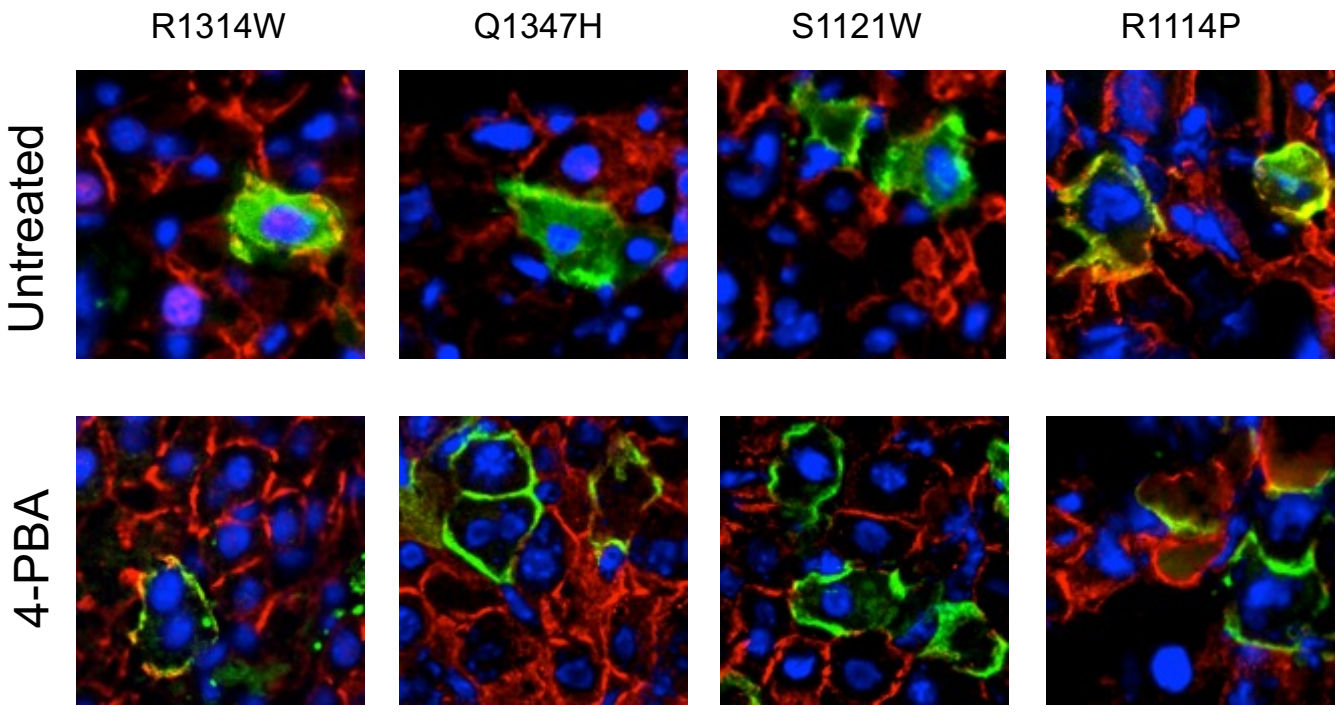


As an existing drug, 4-PBA could be a feasible way to treat PXE patients.

Methods

- Used an ABCC6 KO mouse strain (mice had no ABCC6 protein)
- Hydrodynamic tail vein injections of a plasmid allowed expression of mutant and normal human ABCC6 in liver
- Cryoinjury of hearts induced cardiac calcification in ABCC6 KO mice
- Immunohistochemistry of livers to verify expression of human ABCC6

The cellular localization of 40% of the mutants can be rescued by 4-PBA treatment *in vivo* (in humanized mouse model)



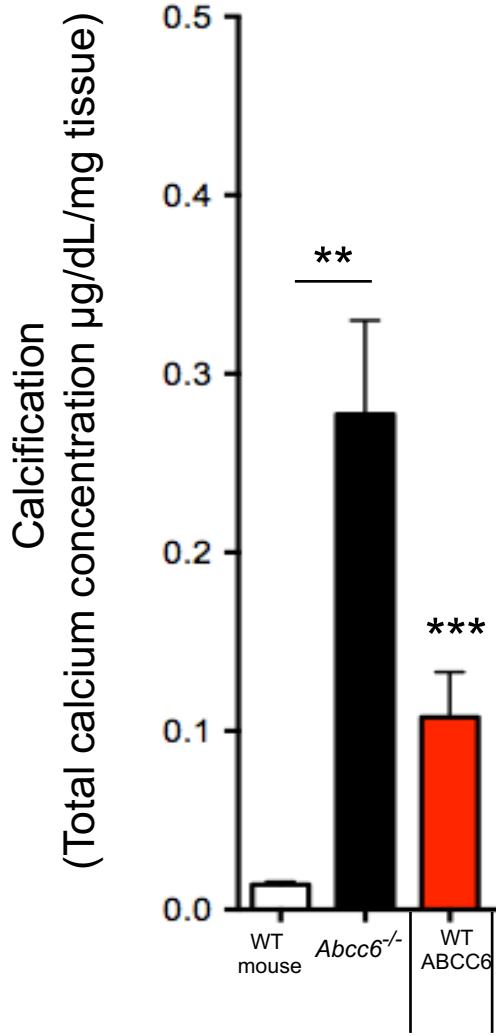
Green: human ABCC6 protein

Red: plasma membrane marker (cadherin)

Blue: nuclei

Pomozi *et al.* 2013, J. Invest. Dermatol.; Le Saux *et al.* 2011, PLoS One

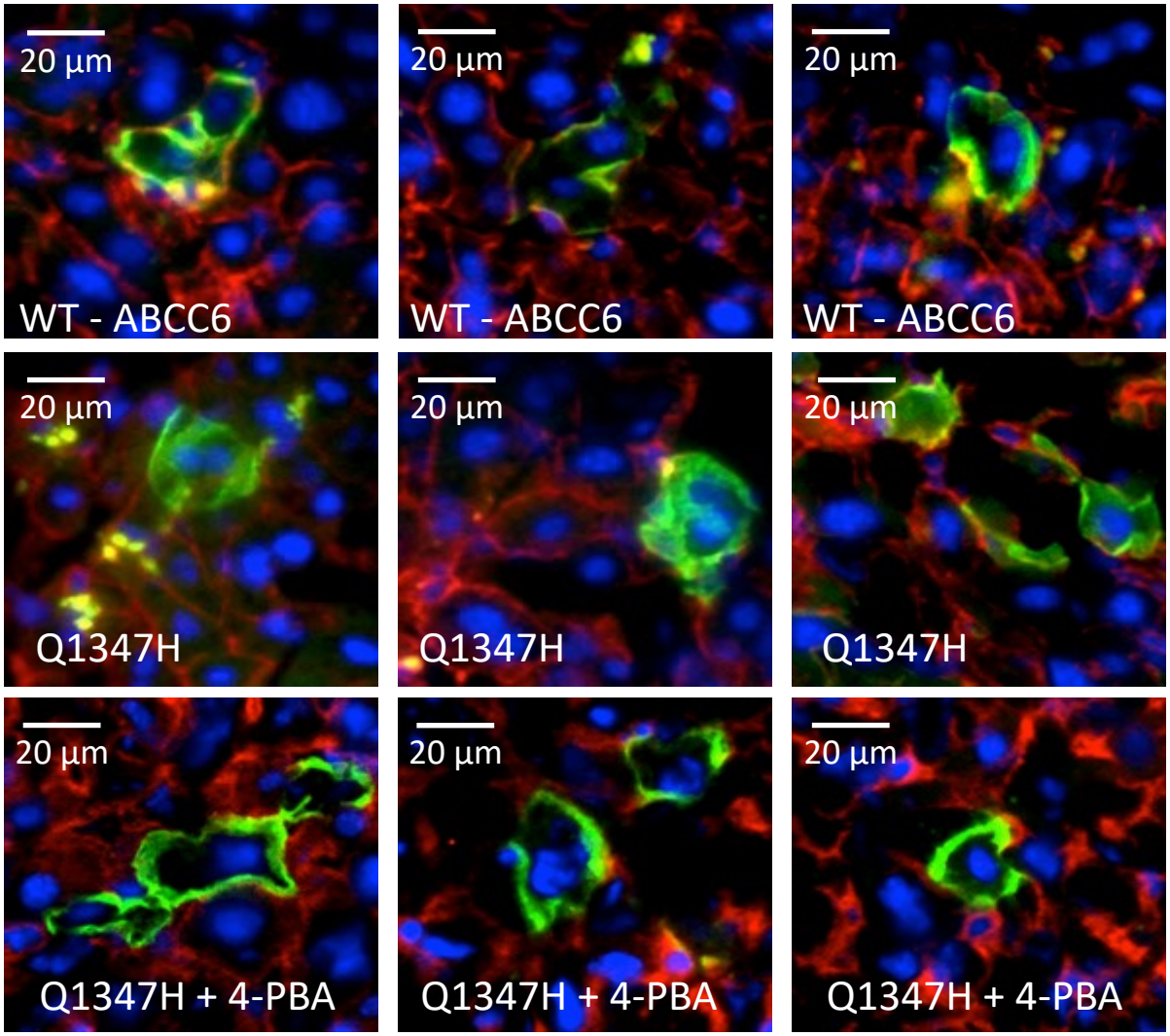
Heart Calcification



My Role in the Project

- These results were submitted for publication in the Journal of Investigative Dermatology
  - Reviewers requested additional data, in particular histology of hearts
- Immunohistochemistry to verify that mouse livers expressed the human *ABCC6*
- Microscopy imaging

My Role in the Project



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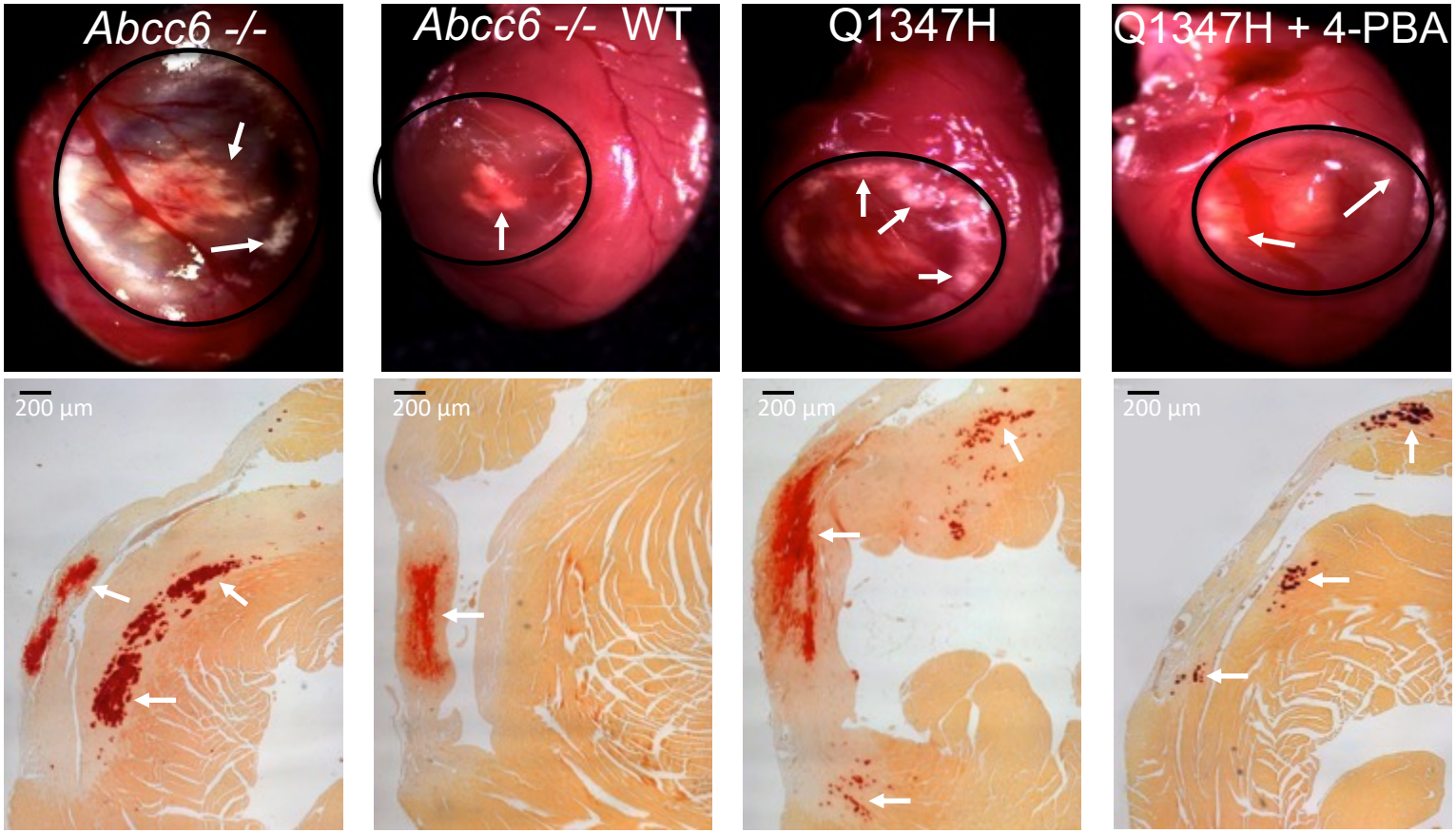
My Role in the Project



Conclusion

- 4-PBA is effective for certain mutants (R1314W, Q1347H, S1121W)
  - Can stop and prevent calcification, but cannot reverse existing calcification
- 4-PBA can be used to treat some PXE patients
- The manuscript was resubmitted

(V. Pomozi, C. Brampton, F. Szeri, D. Dedinszki, E. Kozak, K. van de Wetering, H. Hopkins, L. Martin, A. Varadi, and O. Le Saux)

Acknowledgments