TISSUE-SPECIFIC DOWN REGULATION OF *ABCC6* EXPRESSION IN BETA-THALASSEMIA MICE

Student Researcher: Chloe Edinger
Mentors: Oana Bollt, Olivier Le Saux; University of Hawaii, John A. Burns School of Medicine, Department of Cell and Molecular Biology, Honolulu, HI

INTRODUCTION

The *ABCC6* gene encodes a membrane protein that belongs to the subfamily C of the ABC transporter super family. *ABCC6* is primarily expressed in the liver and kidneys and the encoded protein is associated with the basolateral membrane of hepatocytes and kidney proximal tubules cells. *ABCC6* transports unknown molecules across the plasma membrane, presumably toward the blood stream. Mutations in *ABCC6* cause pseudoxanthoma elasticum (PXE), a rare heritable disorder characterized by the calcification of elastic fibers. The functional relationship between *ABCC6* and elastic fiber calcifications is unknown. Recently, it was found that a large number of patients affected by beta-thalassemia also developed a PXE syndrome identical to the typical inherited PXE. Beta-thalassemia is an inherited hemoglobin disorder caused by mutations in the beta globin gene. Beta-thalassemia patients present a significant reduction in the production of the β-globin chains and severe anemia. Although the PXE manifestations in beta-thalassemia patients are identical to those of inherited PXE individuals, they occur without mutation in the *ABCC6* gene.

We hypothesized that a molecular mechanism separate from genetic alterations alters the expression of *ABCC6* gene or affects the translocation, membrane localization and function of its protein in the liver and/or kidneys as a consequence of the hemoglobin disorder. To address this hypothesis, we used mice models of PXE and beta-thalassemia to measure the mouse *ABCC6* expression, determine the localization of the protein in liver and kidney and whether the beta-thalassemia mice develop PXE manifestations.

METHODS

Twelve wild type (WT) and heterozygous Hbbth-3 beta-globin knockout mice (*Hbb*+/−) mice aged 9 and 12 months were sacrificed to harvest various tissues. Liver and kidney tissues were harvested for RNA extraction. Quantitative RT-PCR (qRT-PCR) was performed using a TaqMan gene expression assay specific to *ABCC6*. qRT-PCR results were normalized to *GAPDH* expression. Liver and kidney tissues were also harvested to prepare frozen sections for immunofluorescence staining using an *ABCC6* (S-20) antibody. Finally, liver, kidney, artery, and whiskers samples were fixed in formalin and paraffin-embedded. Paraffin sections were stained with von Kossa's and Alizarin Red methods to reveal calcium deposits typical of PXE. Tissue samples from an *ABCC6*−/− mouse were also used for control purposes.

RESULTS

Our results showed that expression of *ABCC6* decreased in the livers of the beta-thalassemia mice at 9 and 12 months. Although the level of *ABCC6* expression in the 12 month-old wild type mouse was unusually low, there was only one mouse. Quantification of *ABCC6* in the kidney was also performed using qRT-PCR. In contrast to the expression levels in the livers,

ABCC6 expression in the kidneys was stable or showed a slight increase at 9 months in the Hbb +/− mouse. It is important to note that the level of *ABCC6* expression in the kidney is very low and represents abut 5% of that in the liver. Furthermore, because the *ABCC6* expression was decreased specifically in the liver, the localization of the *ABCC6* protein in the basolateral side of the plasma membrane was examined. In Hbb +/− mice, there were markedly different and patchy staining, suggesting that the localization or translocation of *ABCC6* is altered at both 9 and 12 months of age. No visible changes were seen with kidney tissues.

To evaluate the phenotype of the beta-thalassemia mice, histological stains were performed to investigate calcium deposits. In the whiskers, liver, kidney, and aorta, there was no calcification of elastic fibers in the Hbb +/− mice at age 9 or 12 months.

CONCLUSION

We found a down regulation of *ABCC6* expression in the liver but not in the kidney of a beta-thalassemia mouse model. Although, the beta-thalassemia mouse model does not develop a PXE phenotype, it is reasonable to speculate that the same liver-specific *ABCC6* alterations occurs in human beta-thalassemia patients and causes the development of the PXE phenotype.

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