Tissue-specific Down Regulation of ABCC6 Expression in Beta-thalassemia Mice

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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 beta-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 (Hbbth+/−) 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 about 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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**RESOURCES**