X-linked sideroblastic anemia with ataxia (XLSA/A) is a rare syndromic form of inherited sideroblastic anemia associated with spinocerebellar ataxia, and is due to mutations in the mitochondrial ATP-binding cassette transporter Abcb7. Here, we show that Abcb7 is essential for hematopoiesis and formally demonstrate that XLSA/A is due to partial loss of function mutations in Abcb7 that directly or indirectly inhibit heme biosynthesis.

**Materials and methods**

The generation of a conditionally targeted (“floxed”) allele of Abcb7 (Abcb7fl) has been described previously. A targeted point mutant allele was made by site-directed mutagenesis (QuickChange; Stratagen, La Jolla, CA) of the original gene targeting construct to create a glutamic acid-to-lysine mutation at position 433 (E433K) of the mouse protein corresponding to the hematologically most severe, E433K, human allele. All exons and intron/exon boundaries were resequenced in the mutagenized targeting construct. Newly targeted, homologously recombined embryonic stem cell colonies from the 129S4/SvJ background were analyzed for the mutation by sequencing genomic polymerase chain reaction (PCR) products. Similar phenotypic results were obtained from C57BL/6 chimeric animals produced from multiple independent embryonic stem (ES) cell lines with or without the Neo8 cassette.

C57BL/6-Mxi-Cre animals were obtained from Jackson Laboratories (Bar Harbor, ME) and bred to the previously described Abcb7fl allele on a mixed 129S4/SvJae × 129Sv/EvTac background. F3 animals were used for most experiments. Induction of Cre expression was achieved in 4-day-old or 4-week-old animals by subcutaneous injection of 200 μg or intraperitoneal injection of 400 μg, respectively, polyinosine-polycytosine (pI-pC) on alternate days, for a total of 3 doses. Clinically ill animals were humanely killed in accordance with guidelines accepted by the Animal Care and Use Committee at Children’s Hospital Boston. As a case control, a sex-matched, Mxi-Cre–negative animal injected with pI-pC was phenotyped in parallel with each moribund experimental animal. Complete blood counts, zinc protoporphyrin quantification, and Gpi1 isozyme chimerism analyses were performed as previously described.

For electron microscopy studies, whole blood was processed as previously described. Electron microscopic thin sections were examined with and without lead and uranyl acetate staining on an FEI/Phillips EM 208S (FEI Electron Optics BV, Eindhoven, Netherlands) equipped with an AMT (Advanced Microscopy Techniques, Danvers, MA) digital camera. Light microscopic images were acquired on a Nikon Eclipse E600 microscope with a 100×/0.30 NA oil immersion lens and an RT Slider SPOT 2.3.1 camera (Diagnostic Instruments, Sterling Heights, MI) using SPOT Advanced software (version 3.5.9).

**Results and discussion**

In order to evaluate the effect of Abcb7 deletion on hematopoiesis, we bred the Abcb7fl allele to the interferon-α–inducible Mxi-Cre
line. As previously reported, following induction we found highly variable systemic deletion, including near-complete rearrangement in the bone marrow and liver, no effect in testes or brain, and intermediate levels of rearrangement in all other tissues examined (Figure S1, available on the Blood website; see the “Supplemental Figures” link at the top of the online article). Serial peripheral blood smears demonstrated the presence of a transient population of siderocytes that peaked at day 5 in Abcb7fl + Cre animals (Figure 1A,C and Figure S2); siderocytes were not present in animals without Cre.

Transmission electron micrographs of day-5 peripheral blood showed a subset of reticulocytes from experimental animals with clusters of swollen, pale, damaged mitochondria; electron-dense intramitochondrial deposits, typical of siderocytes, were not seen (Figure 1E). However, treatment of whole blood with acid ferrocyanide iron stain prior to processing the cells for electron microscopy highlighted the inner and outer membranes of abnormal mitochondria in mutant cells (Figure 1G). Consequently, there is iron deposited in mitochondria insufficient to produce electron-dense deposits, but adequate enough to produce a detectable histochemical reaction. Marrow ringed sideroblasts were not present in either group, as assessed by light microscopy iron staining, during the entire time course (data not shown).

At 1 to 2 weeks after the disappearance of siderocytes, Abcb7fl + Cre animals became clinically ill, with signs including lethargy, rubor, epistaxis, and rectal bleeding, necessitating that the animals be humanely killed. The median survival of +Cre newborns was 16 days (Figure 2A and Figure S3). Postmortem gross and microscopic examination typically showed histologic evidence of gastrointestinal, urologic, and/or intracranial hemorrhage with or without microscopic evidence of systemic bacterial infection. Consistent with these findings, blood counts (CBCs) revealed severe pancytopenia (Figure 2B), and the bone marrow was markedly hypocellular (Figure 2D). CBCs taken from animals at day 11 of the induction protocol, prior to the onset of clinical signs, showed that the platelet and white blood counts were less than 25% of control values (Figure 2B). There was partial or near-complete gene deletion in tissues outside the hematopoietic system, but apart from characteristic hepatocellular iron deposits fully described elsewhere, we did not observe gross or microscopic phenotype in other tissues. It is possible that with time we would see other abnormalities, but the uniform and rapidly fatal bone marrow failure phenotype precluded their ascertainment.

In order to circumvent the bone marrow–dependent lethality, we created a targeted point mutant allele corresponding to the hematomically most severe human E433K mutation. Male chimeras were, however, infertile due to testicular atrophy and incomplete spermatogenesis associated with intersitial iron deposition (not shown), which is a phenotype not reported in human subjects with XLSA/A. In other nonhematopoietic tissues, despite substantial chimerism we observed no gross or histologic abnormalities. In particular, animals did not appear ataxic or otherwise neurologically compromised. In the blood, however, a population of siderocytes was present in proportion to the degree of chimerism, as assessed by Gpi1 isozyme analysis (Figure 3A). Similar to the Abcb7fl + Mx1-Cre animals, abnormal mitochondria were readily seen in reticulocytes; however, iron deposition could be demonstrated only by acid ferrocyanide enhancement (Figure 3B-C), and marrow ringed sideroblasts were not visualized by either method (data not shown).

Our ability to characterize the biochemical phenotype of the Abcb7E433K allele was severely restricted by the unexpected male infertility, which precluded germ line propagation. Nonetheless, analysis of chimeric blood demonstrated that the proportion of
Figure 3. An Abcb7/E433K mutation results in siderocytosis with increased zinc protoporphyrin. (A) Siderocytes (arrows) in the peripheral blood of an Abcb7/E433K chimera (Fe stain). Transmission electron micrographs of Abcb7/E433K chimera reticulocytes unstained (B) or with Fe stain (C). Original magnification ×44 000. (D) RBC zinc protoporphyrin-to-heme ratio as a function of Abcb7 E433K chimism. The Abcb7E433K chimera has a significantly higher zinc protoporphyrin level than the wild-type Abcb7 chimera.

References
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Acknowledgments
This work was supported by National Institutes of Health grant DK62474, the Wilkes Fund of the Children’s Hospital Department of Pathology, and the Pew Biomedical Scholars Program (M.D.F.). C.P. was funded in part by L’Association pour la Recherche sur le Cancer (ARC).

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Conflict-of-interest disclosure: The authors declare no competing financial interests.

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Abcb7, the gene responsible for X-linked sideroblastic anemia with ataxia, is essential for hematopoiesis

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