Published in final edited form as:

Blood Cells Mol Dis. 2009 ; 42(1): 1–4. doi:10.1016/j.bcmd.2008.08.001.

Severe Iron Overload with a Novel Aminolevulinate Synthase Mutation and Hepatitis C Infection. A Case Report

Pauline Lee, Ph.D.,

Associate Professor of Molecular Medicine, The Scripps Research Institute, Department of Molecular and Experimental Medicine, 10550 North Torrey Pines Road, MEM 215, La Jolla, 92037, plee@scripps.edu

Lawrence Rice, M.D.,

Chief of Hematology, The Methodist Hospital, Professor of Medicine, Cornell Weill Medical College, 6550 Fannin, Suite 1001, Houston, TX 77030, lrice@tmhs.org

John J. McCarthy, M.D., and

Division of Hematology, The Methodist Hospital, Associate Professor of Medicine, Cornell Weill Medical College, 6550 Fannin, Suite 1001, Houston, TX 77030, jmccarthy@tmhs.org

Ernest Beutler, M.D.

Chairman, Department of Molecular and Experimental Medicine, The Scripps Research Institute, 10550 North Torrey Pines Road, MEM215, La Jolla, CA 92037, beutler@scripps.edu

Abstract

A 55 year old man with a history of chronic hepatic C infection was found to have severe hemochromatosis: hepatic cirrhosis, cardiomyopathy, arrhythmia, hypogonadism, diabetes and bronzed skin color. After 50 phlebotomies, he underwent a combined heart and liver transplant. Genetic analyses identified a novel mutation in the iron responsive element of the *ALAS2* gene. No mutations were found in other genes associated with adult or juvenile hemochromatosis including *HFE*, transferrin receptor-2 (*TfR2*), ferroportin (*SLC40A1*), hepcidin (*HAMP*) and hemojuvelin (*HJV*). We suggest that the *ALAS2* mutation together with chronic hepatitis C infection may have caused the severe iron overload phenotype.

Keywords

ALAS2; iron responsive element; iron overload; hepatitis C; liver transplant; hemochromatosis

Introduction

Erythroid-specific δ -aminolevulinate synthase 2 (*ALAS2*) is the first and rate-limiting enzyme in the heme biosynthetic pathway. Coding region and promoter mutations in *ALAS2*, an X-linked gene, often cause defective or diminished enzyme activity leading to reduced protoporphyrin IX synthesis, accumulation of iron, and insufficient hemoglobin levels [1,2]. The low blood hemoglobin level drives an increase in erythropoiesis causing increased iron

Correspondence to: Pauline Lee.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

uptake and tissue loading. Clinically, patients present with moderate to severe sideroblastic anemia, and hemochromatosis from an early age disproportionate with the number of transfusions received. Males with hemizygous *ALAS2* mutations are often more severely affected but variable penetrance has been reported between brothers with identical mutations [3]. Women heterozygous for *ALAS2* mutations often manifest milder disease, but cases with severe disease have been observed and attributed to skewed X-inactivation [4,5].

ALAS2 is regulated at the translational level by the presence of an iron responsive element (IRE) located in the 5' untranslated region (UTR) of the mRNA [6]. Under low iron conditions, IRE binding proteins (IRPs) bind to 5' IRE's and block translation. Under high iron conditions, iron is bound to the IRP's; the FeS-IRP is then unable to bind to the 5' IRE and translation proceeds normally.

Mutations in 5'IRE's have been associated with disease resulting from overexpression of the unregulated gene. Examples include mutations in the 5' IRE of ferritin light chain, associated with autosomal dominant hereditary hyperferritinemia cataract syndrome [7], and mutation in the 5' IRE of ferritin heavy chain, associated with autosomal dominant hyperferritinemia [8]. Deletion of the 5' IRE in ferroportin in the *Pcm* mutant mouse increases intestinal and hepatic expression of ferroportin, leading to reticuloendothelial iron overload [9].

Modest iron overload has been observed in subjects with chronic hepatitis C infection and end-stage liver disease [10]. Hepatitis C infection should not, in and of itself, lead to severe iron overload of the heart and liver, hypogonadism, diabetes and bronzing of the skin. The association of mutations in the gene responsible for the most common form of primary hemochromatosis, *HFE*, with an increase in the severity of hepatic iron overload and end-stage liver disease in patients with hepatitis C infection is controversial at best [11,12]. Nevertheless, *HFE* hemochromatosis patients with hepatitis C infection generally do not exhibit cardiac iron loading and hypogonadism. In contrast, primary hemochromatosis associated with severe liver and heart iron loading, cardiomyopathy and hypogonadism are more often associated with mutations in juvenile hemochromatosis genes, hemojuvelin (*HJV*) [13], and hepcidin (*HAMP*) [14].

In this report, we describe a patient with chronic active hepatitis C infection, severe liver and cardiac iron overload, hypogonadism, diabetes and bronzed pigmentation. Sequence analyses determined that he was hemizygous for a novel mutation in the 5' IRE of the *ALAS2* gene, and did not have mutations in any known genes associated with primary hemochromatosis: *HFE*, *TfR2*, *Slc40A1*, *HJV* and *HAMP*. This case suggests that mutations in the IRE of *ALAS2* can be associated with iron overload disease, especially if additional risk factors such as hepatitis C are present.

Methods

Human Subjects

The performance of this work was approved by the Internal Review Board for the use of Human Subjects Committee of The Scripps Research Institute. All samples described in this study were obtained with informed consent.

Genetic Screening

Direct sequencing of the genes for *ALAS2*, *HFE*, *TfR2*, *SLC40A1*, *HAMP* and *HJV* were performed as previously described [15–18].

Iron Responsive Element Gel Retardation Assays

K562 cells were treated overnight with deferral (100 μ M) and lysates prepared and used in gel retardation assays. RNA probes were made by transcription with oligos for ALAS2 wildtype: CTTGAACCTAAAGTCCTGTTGCCCTGCACTGAGGACGAACGAATGACAGGTG CGCCCTATAGTGAGTCGTATTA or ALAS2 mut: CTTGAACCTAAAGTCCTGTTG CCCTGCACTGAGAACGAACGAATGACAGGTGCGCCCTATAGTGAGTCGTATT A, ^{32} P UTP, T7 primer and T7 RNA polymerase. Gel retardation was performed in a 20 μ l reaction mix containing the RNA IRE ^{32} P probe (0.1 pmol), 200 μ g K562 cell lysate, 10 mM Hepes pH 7.4, 40 mM KCl, 3 mM MgCl₂, 5% glycerol, 12.5 mM dithiothreitol, 1U RNasin, and the non-radioactive competitor as indicated. Incubation was performed on ice for 15 min and at room temperature for 15 min followed by electrophoresis on a 5% polyacrylamide/0.5x TBE gel.

Case Report

The patient, born in 1950, was found to have hepatitis C in 1980. Brief treatment with interferon and ribavirin in the 1990's was poorly tolerated. In 2003, liver biopsy showed advanced liver disease, and studies revealed massive esophageal varices and moderate splenomegaly. Ascites and encephalopathy emerged in 2004, as did paroxysmal atrial fibrillation. At that time there was "dark bronzed" skin, jaundice, gynecomastia and testicular atrophy. Transferrin saturation was 97%, serum ferritin >1500 ng/ml, and MRI scan by T2* method showed severe iron deposition in liver (7 msec; normal > 19) and heart (10 msec; normal > 20) (Figure 1). EKG showed left anterior fascicular block, poor R wave progression and long QT interval. On echocardiogram and cardiac catheterization, there was global hypokinesis, ejection fraction 21–30%, and normal coronary arteries. Endocrine studies showed hypogonadotropic hypogonadism (LH 1.2, testosterone 140, free testosterone 19.5, all below normal), adrenal insufficiency (AM cortisol <0.1 and PM 7 μ g/dL, ACTH 17 pg/mL) and normal thyroid function. Oral hypoglycemics were started for early diabetes mellitus. Hemoglobin was 13.3 gm/dL, hematocrit 37%, MCV 117, WBC 8100, platelets 61×10^9 /L. Phlebotomy therapy was initiated with darbepoietin to facilitate this. After 8 phlebotomies, the serum iron was 249 μ g/dl and ferritin 1099 ng/ml. Approximately 40 additional phlebotomies were performed prior to combined heart and liver transplantation in August 2005. Since then the patient has done extremely well. After 14 additional phlebotomies, the most recent ferritin was 100 ng/ml. His current MCV is normal (97 fL).

The patient has no personal or family history of significant anemia nor joint problems. There is no family history of hemochromatosis, liver or premature heart problems. His parents are alive in their ninth decade, the father with coronary artery disease and mother with type II diabetes. Two brothers (ages 60 and 58), one sister (age 55) and one son (age 20) are in good health.

Results

The proband was found to have a mutation in the iron responsive element located in the 5' UTR of the *ALAS2* gene (nt –34 C>T, numbering from the start of translation) (Figure 2A). The mutation is located in the stem of the IRE. RNAfold analyses (Vienna RNA Secondary Structure Prediction, www.rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi) predict a loss of the minimum free energy from –12.7 kcal/mol to –9.9 kcal/mol and a loss of free energy of the thermodynamic ensemble from –13.46 kcal/mol to –11.29 kcal/mol but no difference in the predicted structure for the mutant IRE (Figure 2B). Gel retardation assays examining binding of wildtype *ALAS2* IRE to cytosolic extracts demonstrated that the mutant *ALAS2* IRE exhibited slightly lower affinity than wildtype *ALAS2* IRE (Figure 2C). Family members of the proband, the mother, and three siblings were examined for the *ALAS2* mutation. The mother,

sister, and nieces were found to be heterozygous for the *ALAS2* mutation. The mother and sister exhibited no abnormal hematologic findings (data not shown). One brother was found to be hemizygous for the wildtype sequence and the other hemizygous mutant (Figure 2A and D). The hemizygous mutant brother of the patient had no known health problems and no medical information was available.

The patient was also examined for mutations in other genes associated with adult and juvenile hemochromatosis. Specifically, no mutations were identified in *HFE*, $\beta 2$ microglobulin, transferrin receptor-2 (*TfR2*), ferroportin (*SLC40A1*), hemojuvelin (*HJV*) and hepcidin (*HAMP*) genes.

Discussion

ALAS2 mutations are generally located in the coding region and result in diminished enzyme activity. Mutations can vary in phenotype from severe anemia with ringed sideroblasts to severe iron overload with minimal anemia. The mutation reported here is novel since it is located in the iron response element of the *ALAS2* gene. One would predict that such a mutation would not be associated with anemia or ringed sideroblasts, which are caused by insufficient protoporphyrin IX production. Rather, one might predict unregulated, high levels of *ALAS2* expression, possibly with increased heme production. High levels of *ALAS2* or heme might cause a dysregulation of red cell turnover by causing a more rapid progression from proliferating erythroid precursors to terminal differentiation driving increased erythropoiesis and iron uptake.

The patient reported herein had chronic active hepatitis C infection that could predispose to a modest degree of hepatic iron accumulation. The hepatitis cannot explain the massive iron-loading that occurred, suggesting that our identification of the *ALAS2* nt -34 C>T mutation in the stem region of the IRE was pathophysiologically important. Strengthening this case is the fact that no mutations were found in other genes associated with iron overload disease, the genes coding for *HFE*, *TfR2*, ferroportin, hepcidin, and hemojuvelin. Since one brother was hemizygous for the *ALAS2* IRE mutation but without hepatitis C infection, and healthy, we can conclude that mutant genotype alone does not lead to the iron overload phenotype. We conclude that *ALAS2* mutations can be associated with severe clinical iron overload, particularly if additional risk factors such as hepatitis C infection are present.

Acknowledgments

This is manuscript 19317-MEM from the Scripps Research Institute. This work was supported by grants from the National Institutes of Health grant DK53505-09 and the Stein Endowment Fund.

References

1. May A, Bishop DF. The molecular biology and pyridoxine responsiveness of X-linked sideroblastic anaemia. *Haematologica* 1998;83:56–70. [PubMed: 9542324]
2. Bekri S, May A, Cotter PD, et al. A promoter mutation in the erythroid-specific 5-aminolevulinate synthase (*ALAS2*) gene causes X-linked sideroblastic anemia. *Blood* 2003;102:698–704. [PubMed: 12663458]
3. Cazzola M, May A, Bergamaschi G, et al. Absent phenotypic expression of X-linked sideroblastic anemia in one of two brothers with a novel *ALAS2* mutation. *Blood* 2002;100:4236–4238. [PubMed: 12393718]
4. Cazzola M, May A, Bergamaschi G, et al. Familial-skewed X-chromosome inactivation as a predisposing factor for late-onset X-linked sideroblastic anemia in carrier females. *Blood* 2000;96:4363–4365. [PubMed: 11110715]

5. Aivado M, Gattermann N, Rong A, et al. X-linked sideroblastic anemia associated with a novel ALAS2 mutation and unfortunate skewed X-chromosome inactivation patterns. *Blood Cells Mol Dis* 2006;37:40–45. [PubMed: 16735131]
6. Cox TC, Bawden MJ, Martin A, May BK. Human erythroid 5-aminolevulinate synthase: promoter analysis and identification of an iron-responsive element in the mRNA. *EMBO J* 1991;10:1891–1902. [PubMed: 2050125]
7. Cazzola M, Bergamaschi G, Tonon L, et al. Hereditary hyperferritinemia-cataract syndrome: relationship between phenotypes and specific mutations in the iron-responsive element of ferritin light-chain mRNA. *Blood* 1997;90:814–821. [PubMed: 9226182]
8. Kato J, Fujikawa K, Kanda M, et al. A mutation, in the iron-responsive element of H ferritin mRNA, causing autosomal dominant iron overload. *Am J Hum Genet* 2001;69:191–197. [PubMed: 11389486]
9. Mok H, Jelinek J, Pai S, et al. Disruption of ferroportin 1 regulation causes dynamic alterations in iron homeostasis and erythropoiesis in polycythaemia mice. *Development* 2004;131:1859–1868. [PubMed: 15084469]
10. Silva IS, Perez RM, Oliveira PV, et al. Iron overload in patients with chronic hepatitis C virus infection: clinical and histological study. *J Gastroenterol Hepatol* 2005;20:243–248. [PubMed: 15683427]
11. Gattoni A, Parlato A, Vangieri B, et al. Role of hemochromatosis genes in chronic hepatitis C. *Clin Ter* 2006;157:61–68. [PubMed: 16669553]
12. Eisenbach C, Gehrke SG, Stremmel W. Iron, the HFE gene, and hepatitis C. *Clin Liver Dis* 2004;8:775–785. [PubMed: 15464655]
13. Lanzara C, Roetto A, Daraio F, et al. The spectrum of hemojuvelin gene mutations in 1q-linked juvenile hemochromatosis. *Blood* 2004;103:4317–4321. [PubMed: 14982873]
14. Roetto A, Papanikolaou G, Politou M, et al. Mutant antimicrobial peptide hepcidin is associated with severe juvenile hemochromatosis. *Nat Genet* 2003;33:21–22. [PubMed: 12469120]
15. Lee PL, Halloran C, West C, Beutler E. Mutation analysis of the transferrin receptor-2 gene in patients with iron overload. *Blood Cells Mol Dis* 2001;27:285–289. [PubMed: 11358389]
16. Lee PL, Beutler E, Rao SV, Barton JC. Genetic abnormalities and juvenile hemochromatosis mutations of the *HJV* gene encoding hemojuvelin. *Blood* 2004;103:4669–4671. [PubMed: 14982867]
17. Lee PL, Gelbart T, West C, et al. A study of genes that may modulate the expression of hereditary hemochromatosis: Transferrin receptor-1, ferroportin, ceruloplasmin, ferritin light and heavy chains, iron regulatory proteins (IRP)-1 and -2, and hepcidin. *Blood Cells Mol Dis* 2001;27:783–802. [PubMed: 11783942]
18. Barton JC, Lee PL. Disparate phenotypic expression of ALAS2 R452H (nt 1407 G --> A) in two brothers, one with severe sideroblastic anemia and iron overload, hepatic cirrhosis, and hepatocellular carcinoma. *Blood Cells Mol Dis* 2006;36:342–346. [PubMed: 16540354]

Figure 1.

Figure 1a: Histology of the heart and liver showing severe iron loading (20x magnification; Prussian blue). 1b: MRI scans before and after transplantation. The markedly attenuated T2-weighted signal intensity of heart and liver revert to normal post-transplant

Figure 2. Genetic and Functional Analyses of the *ALAS2* Mutation Identified in the Proband and His family. A. Sequence analyses showing the hemizygous nt-34C>T mutation in the patient, heterozygous mutation in the patient's mother and normal wildtype sequence of his brother. B. Schematic drawing showing the location of the mutation in the IRE stem loop structure. C. Electrophoretic mobility shift analyses of the wildtype iron responsive element in the presence of 1, no competitor; 2, 100x competitor; 3, 50x competitor; 4, 25x competitor; 5, 12.5x competitor; 6, 6.25x competitor. D. Pedigree of the family of the proband with *ALAS2* genotype shown.