increased activity of the trimeric NF-Y complex. Therefore, the size of the pool of immature hematopoietic cells may be regulated through (de-)activating NF-Y.

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Congenital sideroblastic anemia associated with germline polymorphisms reducing expression of *FECH*

The sideroblastic anemias (SAs) are disorders of ineffective erythropoiesis, collectively characterized by abnormal Prussian blue-positive granules (i.e., iron-stuffed mitochondria) that encircle marrow erythroblast nuclei to form *ringed sideroblast* cells.[1] SAs are usually acquired, but occasionally congenital. While the causes of the common acquired forms of SA remain largely unknown, the molecular genetics of several of the inherited forms of SA is now well understood.[2,3] For instance, X-linked SA is often associated with germline mutations in the erythroid-specfic isoform of 5-aminolevulinate synthase (ALAS2), and several mitochondrial metabolic defects have also been linked to inherited SAs. However, there are still many congenital SA cases of unknown molecular origin.

The precise relationship between SA and erythropoietic protoporphyria (EPP, MIM #177000) is unclear. A substantial fraction of patients with EPP have anemia (48% of women and 33% of men in the largest series), which is usually mild and associated with diminished iron stores.[4] Ferrochelatase, the enzyme deficient in EPP, is encoded by the *FECH* locus at 18q21.3 and catalyzes the final step in heme biosynthesis: addition of ferrous iron to the protoporphyrin ring.[5] In one analysis of 9 EPP patients, scattered ringed sideroblasts were observed by light microscopy in the bone marrows of 7 patients, while mitochondrial electron energy-loss spectroscopy (EELS) indicated SA-like iron compounds in all 9 samples.[6] Additionally, a 1973 report described a case of EPP with fatal liver disease associated with SA-like features.[7] Despite these observations, most idiopathic acquired SA cases do not have *FECH* mutations, even though modest elevations of erythrocyte protoporphyrin levels are common in this group.[3,8]

Here we describe a child who presented with congenital SA of unclear etiology, in whom we detected marked-

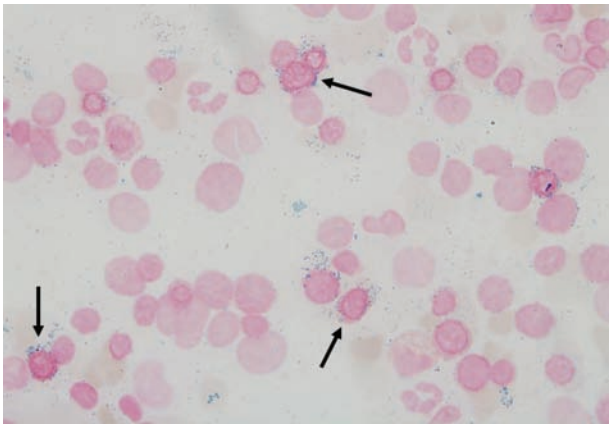

Figure 1. Iron stain of bone marrow aspirate demonstrating ringed sideroblasts. Numerous ringed sideroblasts (arrows) comprising 70-80% of the bone marrow erythroid cells are evident. (Prussian blue reaction, 400X, obtained with Olympus BX 40 microscope (Olympus, Tokyo, Japan) equipped with an Uplan 100 x/1.30 NA oil apochromatic lens and Olympus Q-color 3 CCD camera. Image processed for color balance using Adobe Photoshop CS2 (Adobe Systems, San José, CA, USA).

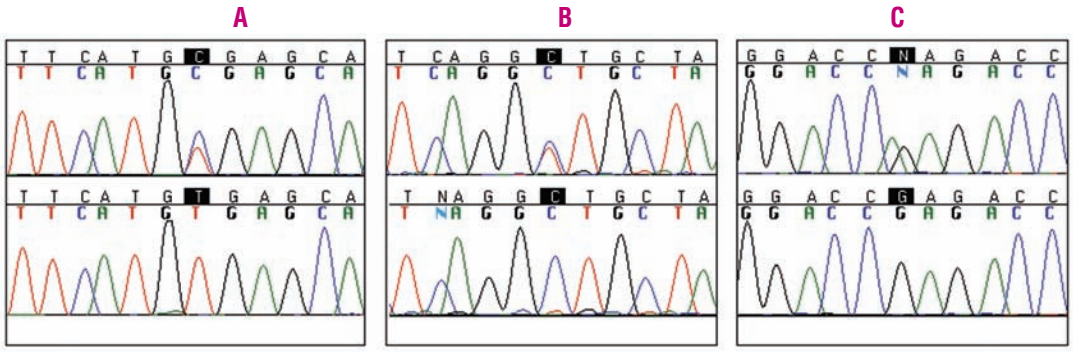

Figure 2. Fluorescent dye chemistry sequencing chromatograms of genomic DNA from the patient (top) and the patient's phenotypically normal mother (bottom). In addition to the commonly-encountered IVS1-23C/T (A) and IVS3-48T/C (B) mutations, the patient also demonstrated a 425G/A [R96Q] polymorphism (C).

ly elevated protoporphyrin and reduced *FECH* mRNA expression compared to healthy controls.

A boy of mixed European descent (age 2 years, 11 months) with an unremarkable perinatal history was noted to be anemic during a well-child evaluation (hemoglobin 9.6 g/dL, mean corpuscular volume 89 fL, and RDW 23.9%). Peripheral smear showed only anisocytosis; white count, leukocyte differential, platelet count, iron studies, and hemoglobin electrophoresis were all unremarkable. Bone marrow examination revealed mild erythroid hyperplasia and 30% ringed sideroblasts. The patient's mother was healthy, and the father was unavailable for study. During a follow-up visit at age 7 years, 8 months, the patient reported a *burning pain* in his hands with sun exposure, but without any erythroderma or blistering. Both plasma and free RBC protoporphyrin was measured and found to be markedly elevated (7.7 µg/dL [normal <1.0 µg/dL]; and 1460 µg/dL [normal <60 µg/dL] respectively). Marrow examination at age 14 showed 80% ringed sideroblasts (Figure 1) with a normal karyotype. Mitomycin C chromosome stress testing was negative. Peripheral blood indices from the date of last follow-up at age 15 revealed a hemoglobin of 9.5 g/dL, mean corpuscular volume of 89.3 fL, and a RDW of 32.9%. Iron studies revealed a serum iron of 138 µg/dL, a total iron-binding capacity of 312 mg/dL, and a ferritin of 45 mg/L (normal 14–336 µg/L).

After obtaining the consent of the patient and his mother for analysis of their biological material and case report, genomic DNA was extracted from peripheral blood mononuclear cells and *FECH* was analyzed as described.[3,9]

Total RNA was isolated from whole peripheral blood from the patient, his mother (maternity confirmed using a VNTR panel), and 2 healthy controls. We performed real-time quantitative polymerase chain reaction (RQ-PCR) using TaqMan© Universal Master Mix, an ABI 7900 Fast™ RT-PCR system, and the Hs00164616_m1 *FECH* FAM primer-probe set (all Applied Biosystems, Foster City, CA, USA). Assays were performed in triplicate, with expression ratios calculated using the $2^{-\Delta\Delta C^T}$ method.

Genomic DNA analysis of the patient revealed heterozygosity for the common promoter -251G, IVS1-23C>T and IVS3-48T>C polymorphisms (*GTC haplotype*) that down-regulate *FECH* expression, as well as a neutral 425G/A (p.R96Q, SNP rs1041951) polymorphism (GenBank accession NP_000131, Figure 2). The patient's mother demonstrated the IVS1-23C>T and IVS3-48T>C polymorphisms. *FECH* mRNA expression in the patient was approximately 40% of that of his mother (20% of normal), whose gene expression in turn was approximately 50% that of healthy controls.

The mutations responsible for the clinical phenotype of EPP are diverse, with no clear correlation between genotype and either protoporphyrin levels, disease severity, clinical phenotype (i.e., liver versus cutaneous disease) or FECH enzyme activity. Inheritance patterns of EPP are complex. For an individual to manifest clinical symptoms of EPP, inheritance of either two mutant alleles (recessive pattern) or both a mutated allele and a low-expression *normal* allele (e.g., the GTC haplotype in this case; dominant pattern with incomplete penetrance) appears to be necessary.[10,11] This case is of interest because of the unusual clinical presentation of EPP dominated by SA (30% and later 80% ringed sideroblasts), rather than photosensitivity (minimal) or hepatic abnormality (absent). To our knowledge this is the first description of a FECH mutation presenting initially as isolated congenital SA, suggesting that EPP should be considered in the differential diagnosis of SA without other features. Similar phenotypic diversity has been described with germline mutations in many other genes. One example that includes porphyria is the GATA1 erythroid transcription factor, where different mutations can lead to thalassemia, macrothrombocytopenia, or congenital erythropoietic porphyria (CEP).[12]

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Correlation of liver iron concentration determined by R2 magnetic resonance imaging with serum ferritin in patients with thalassemia intermedia

Thalassemia intermedia is a highly diverse group of thalassemia syndromes associated with anemia and a range of specific complications, such as extramedullary hematopoiesis, leg ulcers, gallstones and a hypercoagulable state, which are uncommon in patients with thalassemia major.[1] The degree of anemia present in patients with thalassemia intermedia is typically mild and generally does not require regular blood transfusion therapy. However, patients can still be at risk of the clinical sequelae of iron overload (as commonly seen in regularly transfused thalassemia major patients) due to increased intestinal iron absorption triggered by chronic anemia, ineffective erythropoiesis and, possibly, decreased serum hepcidin.[2,3] The principal methods of determining body iron levels are measurement of serum ferritin levels and assessment of liver iron concentration from biopsy tissue. Non-invasive approaches for determining liver iron concentration are increasingly used as an alternative to biopsy, although R2 magnetic resonance imaging (MRI) is currently the only validated approach.[4,5] A significant correlation between serum ferritin and liver iron concentration has been established in regularly transfused patients with thalassemia major.[6,7] Data of patients with thalassemia intermedia are limited, but recent studies have highlighted differences compared with the studies performed in thalassemia major patients.[3,8] In these studies, serum ferritin levels were seen to be significantly lower in patients with thalassaemia intermedia than in those with thalassemia major, despite comparable liver iron concentration (as evaluated by biopsy or superconducting quantum interference device). The aim of our study was to investigate the correlation between liver iron concentration determined by R2 MRI and serum ferritin levels in patients with thalassemia intermedia. The data reported here represent the largest investigation of this correlation in thalassemia intermedia using R2 MRI and, therefore, provide valuable information on the relationship between these parameters in this specific patient population.

This was a cross-sectional study of randomly selected thalassemia intermedia patients treated at a chronic care center in Hazmieh, Lebanon. The sampling frame consisted of 120 thalassemia intermedia patients ≥2 years of age. We were able to contact 109 of these patients by telephone and 74 agreed to participate. Patient charts were reviewed and a medical history compiled, which included details of drug history, co-morbid illnesses and transfusional history. Data from a randomly selected population of patients with thalassemia major treated at the center were also obtained for comparative evaluation. Blood samples were obtained for assessment of serum ferritin levels. Direct determination of iron burden was performed using R2 MRI to obtain liver iron concentration values, using established methodology.[9] The reading of MRI results was performed by Dr. Tim St Pierre. Written informed consent was provided by all patients. Data from 74 thalassemia intermedia patients were included in the analysis (Table 1). Transfusion-naïve patients had significantly lower iron levels compared to those with a history of transfusion therapy (p=0.003). None of the patients were receiving iron chelation therapy at the time of data collection and had not received chelation therapy for at least two years prior to study entry. In addition, none of the patients involved were

Table 1. Patients' characteristics.

	Patients' characteristics
Patient number	n=74
Mean age, ±SD, in years (range)	26.5±11.5 (8-54)
Male/female	33/41
Splenectomized, (%)	59 (79.7)
Mean hemoglobin, g/dL±SD (range)	8.43±1.86 (4.90-13.10)
Transfusion history	
Naïve	20
Transfused	54
Mean SF±SD, ng/mL (range)	1023±780 (15-4140)
Splenectomized	1201±764
Non-splenectomized	428±495
Transfusion-naïve	567.8±455.2
Transfused	1209±429
Mean LIC±SD, mg Fe/g dw (range)	9.0±7.4 (0.5-32.1)
Splenectomized	10.5±6.8
Non-splenectomized	3.9±7.4
Transfusion-naïve	4.0±3.3
Transfused	11.55±7.00

SF: serum ferritin; LIC: liver iron concentration; dw: dry weight.