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Case report

Identification of a ferrochelatase mutation in a Chinese family with erythropoietic protoporphyria☆

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***Background/Aims*: Erythropoietic protoporphyria (EPP) is a rare autosomal dominant disorder of heme biosynthesis characterized by a partial decrease in ferrochelatase (FECH) activity leading to excessive accumulation of protoporphyrin. While a majority of EPP patients only exhibit photosensitivity, a small percentage of patients also develop liver complications and need liver transplantation.**

***Methods*: In this study, we have sequenced the ferrochelatase gene of a Chinese EPP patient who suffered from EPP-related liver complications.**

***Results*: A nonsense mutation in exon 4, 343C>T, introducing a premature stop codon at position arginine 115, was identified in the proband as well as her symptomatic mother and brother, but was absent in her father. All the family members with overt photosensitivity also carried the low-expressed allele IVS3-48c, whose prevalence in the Chinese Han population was determined to be 41.35% and which was also functional in producing an aberrant 63 bp insertion.**

***Conclusions*: We describe the first FECH mutation identified in the Chinese Han population and report a high frequency of the hypomorphic IVS3-48c allele in China.**

***Keywords*: Erythropoietic protoporphyria; Liver complication; Ferrochelatase mutation; IVS3-48T/C**

1. Introduction

Erythropoietic protoporphyria (EPP; MIM177000) is an inherited disorder of heme biosynthesis, caused by partial defects of ferrochelatase (FECH; E.C.4.99.1.1), the terminal enzyme of the heme biosynthetic pathway, which catalyzes the insertion of iron into protoporphyria IX to form the heme molecule. Defective FECH leads to the accumulation of free protoporphyrin in erythrocytes, plasma, skin and liver. Protoporphyrin IX deposition in the skin causes an extremely painful photosensitivity in patients which starts early in childhood [1]. A minority of less than 5% of patients develop various liver diseases from cholestasis to unpredictable liver cirrhosis due to

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hepatic accumulation of protoporphyrin IX. If jaundice develops, a rapidly fatal outcome often follows, unless liver transplantation is undertaken [2,3].

The human FECH gene, located on chromosome 18q21.3 [4], contains 11 exons and 10 introns with its exon/intron boundaries conforming to the consensus donor (GTn) and acceptor (nAG) sequences. FECH cDNA has an opening reading frame of 1269 bases, encoding 423 amino acid residues. The mature protein has a molecular weight of 40–42 kDa, and acts in situ as an 82–84 kDa homodimer.

EPP is marked by high allele heterogeneity as many different FECH gene mutations have been identified. Most individuals with a heterozygous mutation are asymptomatic, despite their decreased levels of FECH activity being only half of the normal value. For protoporphyrin to accumulate sufficiently to cause skin photosensitivity, FECH activity has to fall below a critical threshold of ~35% of the normal level, with the lowest activity observed in patients with advanced EPP liver diseases [5,6]. In most cases, the inheritance of EPP is described as an autosomal dominant disorder with incomplete penetrance. Gouya et al. [7] have recently demonstrated that patients usually share a hypomorphic allele that is common in the general population with low expression in trans due to a rare loss-of-function allele. They identified a common intronic single nucleotide polymorphism (SNP), IVS3-48T/C, and showed that this SNP was responsible for the low expression of the hypomorphic allele due to an aberrantly spliced mRNA causing a 63-bp insertion and responsible for a lower steady level of normal mRNA [8].

EPP cases have been reported in Europe [9,10], the United States [11] and Japan [12,13]. The estimated prevalence of symptomatic EPP among Europeans is approximately 1 in 75,000–200,000 individuals, while a much higher prevalence is found in Southeast Asian countries. In China, EPP is not commonly diagnosed and so far epidemiologic data are unavailable. Only about 100 patients with known EPP have been reported in a country with a population of 1.3 billion [14], but no genetic data are available for these patients. In this article, we report the first EPP family in Chinese Han population with a recurrent FECH mutation. This observation extends the inheritance model of EPP and provides new information elucidating genetic mechanisms responsible for EPP.

2. Patients and results

2.1. Characteristics of patients and family members

The proband is a 31-year-old Chinese female with cutaneous photosensitivity which started at the age of five. She was admitted to Ruijin Hospital, Shanghai Jiaotong University, School of Medicine, on March 2006, complaining of severe abdominal pain, nausea, vomiting, jaundice, in addition to painful, red, swollen face and hands. Two weeks before her admission, she had been treated with transfusion because of dysfunctional uterine bleeding. On examination, she had tender erythema and edema of her face. Laboratory examination showed ALT 172 IU/L (<60 IU/L); AST 149 IU/L (<42 IU/L); AKP 297 IU/L (<121 IU/L); γ-GT 180 IU/L (<64 IU/L); T-Bil 369 μmol/L (<24 μmol/L); D-Bil 229.8 μmol/L (<6.8 μmol/L). Routine blood test showed hemoglobin 77 g/L (>110 g/L); RBC 2.86×10^{12}/L ($>3.5\times10^{12}$/L), and normal leukocyte and platelet count. Serological tests for HAV, HBV, HCV, HEV and HIV were negative. Hepatomegaly and splenomegaly were indicated by MR imaging and ultrasonic examination. Protoporphyrin in red cells was markedly elevated (details in Table 1). The patient was treated with anodyne, diammonium glycyrrhizinate, and other Chinese traditional herbal drugs and recovered one month later. One year later, the patient had a mild elevated ALT and γ-GT level and returned to normal after treatment with Chinese traditional medicine. Her mother and brother also had cutaneous signs of EPP since early age without abnormal liver function (details in Table 1). Her 9-year-old son had no skin photosensitivity or liver disease but had a mild elevated protoporphyrin. The father of the proband and paternal relatives had no sign of EPP. Written consents for genetic analysis were obtained from the patient and her family members according to the guidelines of the Ethics Committee of Shanghai Jiaotong University.

2.2. Genomic DNA extraction and analysis

Genomic DNA from 6 family members was extracted from peripheral blood leukocytes using a Genomic DNA Purification Kit (PUREGENE, Genetra, MN, USA) according to the manufacturer's instructions. All coding regions, the intron/exon junctions with 50–300 bp flanking sequence, were PCR amplified and subjected to direct sequencing using the Big-Dye Terminatior Chemistry and ABI377 automated DNA sequencer (Applied Biosystems, Foster City, CA, USA). The presence or absence of a mutation was confirmed by sequencing both strands.

Human genomic DNA and cDNA is numbered according to the reference sequences (GenBank Accession Nos. AJ250235 and NM000140), and the A of the ATG initiation codon is shown as "+1". As shown in Fig. 1a, the intragenic SNPs of the FECH gene in this family were as follows: −164 in the promoter, rs2272783 (IVS3-48T/C) in intron 3, rs536765 (798C/T) in exon 7, rs536560 (921G/A) in exon 9 and rs8339 (1520C/T) in the uncoding region of exon 11.

Table 1
Clinical, biochemical and genetical findings in the Chinese EPP family

Subjects	Age	Clinical symptoms	ZnPP <1.25 μmol/L	R115X	IVS3-48T/C
Proband (III-M)	31	Photosensitivity, liver complication	19.7↑	TC	CC
Grandmother (I-1)	75	None	ND	CC	TC
Mother (II-1)	58	Photosensitivity	2.6↑	TC	CC
Father (II-2)	59	None	ND	CC	CC
Brother (III-2)	29	Photosensitivity	2.58↑	TC	CC
Husband (III-3)	33	None	1.14	ND	ND
Son (IV-1)	9	None	1.55↑	CC	TC