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The Ferrochelatase Gene Structure and Molecular Defects Associated with Erythropoietic Protoporphyria

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Ferrochelatase [heme synthase, protoheme ferrolyase (EC 4.99.1.1)], the terminal enzyme of the heme biosynthetic pathway, catalyzes the incorporation of ferrous ion into protoporphyrin IX to form protoheme IX. The genes and cDNAs for ferrochelatase from mammals and microorganisms have been isolated. The gene for human ferrochelatase has been mapped to chromosome 18q 21.3 and consists of 11 exons with a size of about 45 kilodaltons. The induction of ferrochelatase expression occurs during erythroid differentiation, and can be attributed to the existence of the promoter sequences of erythroid-related genes. Analysis of the ferrochelatase gene in patients with erythropoietic protoporphyria, an inherited disease caused by ferrochelatase defects, revealed that molecular anomalies of ferrochelatase from 11 patients were found in 9 patients as autosomal dominant type, and 2 patients as recessive type. Diversity of the mutations of the ferrochelatase gene is also briefly described.

KEY WORDS: Ferrochelatase; gene; heme synthesis; erythropoietic protoporphyria; erythroid differentiation.

INTRODUCTION

Ferrochelatase [protoheme ferrolyase (EC 4.99.1.1)], the last-step enzyme of the heme biosynthetic pathway, catalyzes the insertion of ferrous ion into protoporphyrin IX to produce protoheme. The understanding of the enzyme at a molecular level is a prerequisite to elucidating the regulation of iron and heme metabolism. The enzyme is located in the inner membrane of mitochondria of a variety of mammalian cells, and faces the active site to the matrix. cDNAs encoding ferrochelatase from mouse, human, and plants, and the genes from yeast and bacteria have been isolated, and the derived amino acid sequences show 10–88% identity among species. Expression of ferrochelatase seems to occur in all living cells. Mammalian ferrochelatase is markedly induced at the transcriptional level during erythroid differentiation, when iron uptake by cells and hemoglobin synthesis are up-regulated. The induction seems to be controlled by the characteristic promoter of erythroid-related genes. Once the gene for mammalian ferrochelatase is characterized, the molecular basis and clinical diagnosis of erythropoietic protoporphyria (EPP), caused by a deficiency of ferrochelatase, will be developed. We here describe the structure and expression of the ferrochelatase gene. Molecular characterizations of EPP are also shown.

THE GENE STRUCTURE OF HUMAN FERROCHELATASE

Human ferrochelatase mRNA is encoded by a single nuclear gene which spans approximately 45 kb of chromosomal DNA. Isolation of the ferrochelatase gene shows the location to be chromosome 18 q 21.3 (Brenner *et al.*, 1992; Taketani *et al.*, 1992). No biotin-labeled segment of the human ferrochelatase gene is detected in other regions of the chromosomes, confirming that ferrochelatase is encoded by a single gene. The human ferrochelatase gene consists of 11 exons which range in size from 108 to 1293 bp. Of ten introns of the gene, intron 1 is relatively long (~ 7 kb), and a polymorphic nucleotide repeat sequence unique to intron 2 is found, suggesting that

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these may be related to the mode of transmission of EPP (Whitcombe *et al.*, 1991). A pseudogene (~ 30 kb) related to the functional gene for human ferrochelatase has bene isolated (Whitcombe *et al.*, 1994). The pseudogene shows > 80% overall nucleotide sequence identity to the functional gene, but contains no intronic sequences in the region corresponding to the open reading frame of ferrochelatase. Furthermore, the pseudogene contains small deletions and insertions, creating frame-shifts and numerous termination codons; this indicates that it does not encode a functional polypeptide. Location of the pseudogene is mapped to chromosome 3p22–23. The existence of the pseudogene has practical implications for the molecular analysis of mutations responsible for EPP.

The results of Northern blot analysis indicate that the gene encodes two mRNAs of 2.5 and 1.6 kb, which are present in all cells including human leukemia HL60 cells, K562 cells, hepatoma HepG2 cells, and Epstein–Barr virus-transformed lymphoblastoid B cells (Nakahashi *et al.*, 1990b, 1992). The two bands of human ferrochelatase mRNA are attributed to two polyadenylation sites (Nakahashi *et al.*, 1990b). This is similar to the transcript of the mouse ferrochelatase gene, which appears as a 2.2- and 2.9-kb mRNA in various tissues (Taketani *et al.*, 1990; Chan *et al.*, 1993). When murine erythroleukemia (MEL) cells are induced to differentiate with dimethylsulfoxide (DMSO), the amounts of ferrochelatase mRNA increase 4- to 7-fold by 72 h, and the size of the mRNA in the differentiated MEL cells is the same as that in nonerythroid cells (Taketani *et al.*, 1990; Chan *et al.*, 1993). Northern blot analysis of rat ferrochelatase mRNA reveals that the ferrochelatase mRNA in liver is shown to be a single 2.4-kb band, and that the amount of an mRNA of the same size markedly increases in the spleen of anemic rats (Taketani, 1993). Based on the above observations, the structure of the transcript of ferrochelatase in erythroid cells appears to be identical to that found in nonerythroid cells.

Primer extension analsysis indicates that ferrochelatase mRNA in human erythroleukemia K562 cells has the same 5′ terminus as that in human hepatoma HepG2 cells (Taketani *et al.*, 1992). The size of the MEL cell enzyme (41 kDa), as determined by sodium dodecylsulfatepolyacrylamide gel electrophoresis, appears to be identical to that of mouse liver (Nakahashi *et al.*, 1990a). In addition, peptide mapping of the enzyme from liver, as well as that from MEL cells treated with DMSO, shows identical digestion peptides, suggesting that only one type of ferrochelatase is expressed in erythroid and nonerythroid tissues (Nakahashi *et al.*, 1990a). Thus the regulation of ferrochelatase enzyme expression is different from the mechanism by which tissue-specific expressions of porphobilinogen (PBG) deaminase is controlled, which utilizes 5′-alternative splicing of erythroid-specific promoter situated 3′ to the housekeeping promoter (Chretien *et al.*, 1988). The 5′-flanking sequence of the human ferrochelatase gene reveals that it does not contain the CAAT or TATA box but the GC box, indicating that the gene is responsible for the maintenance of normal housekeeping functions. Bacterial chloramphenicol acetyltransferase assay revealed that the upstream region between positions -263 and -32 contained cis-acting elements that are essential for basic expression of the human ferrochelatase gene in HeLa cells (Taketani, unpublished data), and this suggests that the promoter recognizing a transcriptional factor Sp1 may be sufficient for the expression. Taking these observations together with the structure of the gene, mRNA, and protein, this gene is found to be active in all animal cells. The 5′-promoter region of the human ferrochelatase gene contains a possible GATA-1 binding site at position -321 to -316 (ATATCT), in the opposite orientation, which conforms with 5 out of 6 bases to the consensus sequence (WGATAR: W = A/T and R = A/G) (Taketani *et al.*, 1992). Furthermore, the consensus sequence related to the GATA-1 binding site is found at positions -566 to -561 and -561 to -556. These DNA motifs serve in part as a signature for erythroid-specific gene expression and are present in the promoters of the globin gene (Orkin 1990) and of genes for erythroid-inducible enzymes for heme biosynthesis.

The sequence TGAGTCA, which is the consensus sequence of the NF-E2 binding site, is found at positions -277 to -271 (Taketani *et al.*, 1992). The NF-E2 motif was first demonstrated in the erythroid-type PBG deaminase promoter (Mignotte *et al.*, 1989a; Philipson *et al.*, 1990), and more recently, multiple sites have been found in the upstream enhancer of the β-globin gene (Mignotte *et al.*, 1989b). Among the genes for the heme biosynthetic enzymes, the NF-E2 sequence is also found in the human erythroid 5-aminolevulinic acid (ALA) synthase gene (Yamamoto *et al.*, 1994). A gel retardation assay revealed that specific proteins in nuclear extracts of MEL cells bind to the -381/-261 segment of the 5′-promoter

region of the ferrochelatase gene (Taketani, 1993). Competition experiments demonstrated that one of the MEL nuclear extract proteins was competed off with an oligonucleotide containing the GATA-1 binding site (-329/-307), and that another protein binding was diminished by excess probe containing the NF-E2 binding site (-289/-264). The gene encoding p45 NF-E2 has been mapped to mouse chromosome 15 near the mutation microcytosis (*mk*) (Peters *et al.*, 1993a). The *Mk/mk* mouse, which is severely anemic due to defects intrinsic to both hematopoietic cells and intestinal cells, showed decreased expression of ferrochelatase (< 50%) in reticulocytes (Peters *et al.*, 1993b). These results suggest that NF-E2 cofunctions with GATA-1 to play a role in the induction of ferrochelatase during erythroid differentiation. Since recent studies (Igarashi *et al.*, 1994) suggest that an additional regulatory step could be provided by association of p45 NF-E2 with different members of the *maf* family of protooncogenes, additional regulatory mechanisms may be involved in the induction of ferrochelatase expression in erythroid cells.

REGULATION OF THE GENE EXPRESSION IN ERYTHROID CELLS

Regulation of heme biosynthesis is different in hepatic (nonerythroid) and erythroid tissues. In nonerythroid cells, the rate of heme synthesis is controlled by the first pathway enzyme, ALA synthase, at transcriptional and post-transcriptional levels (Yamamoto *et al.*, 1994). In differentiating erythroid cells, the housekeeping ALA synthase does not have the same regulatory role, and a second ALA synthase isozyme (i.e., erythroid specific) is present (Yamamoto *et al.*, 1994). Differentiation occurs after ferrochelatase is induced, even though the other enzymes of the heme biosynthetic pathway are induced earlier. Mutationally or chemically induced blockage of ferrochelatase arrests the differentiation (Rutherford *et al.*, 1979; Fadigan and Dailey, 1987). Thus, it has been considered that ferrochelatase could play a role in the rate-limiting step of differentiation of erythroid cells. Analysis of the ferrochelatase gene shows that the gene is present in single copy in the genome and that one type of transcript is produced in erythroid and nonerythroid cells. The 5′-flanking region of the human ferrochelatase gene has binding sites for erythroid-specific transcriptional factors (GATA-1 and NF-E2), which may regulate the induction of ferrochelatase during erythroid differentiation. An increase of ferrochelatase mRNA in MEL cells is observed within 12 h after the addition of DMSO and parallels those of erythroid ALA synthase (Fukuda *et al.*, 1993a), coproporphyrinogen oxidase (Kohno *et al.*, 1993), and protoporphyrinogen oxidase (Taketani, unpublished). These inductions are earlier than those of mRNAs encoding other heme biosynthetic enzymes (Fujita *et al.*, 1991). The findings suggest that the concomitant increase in the expressions of the terminal enzymes of heme biosynthesis and erythroid ALA synthase may be the important events in erythroid differentiation. Synthesis of ferrochelatase can be regulated at the post-transcriptional level, since the stability of ferrochelatase mRNA is regulated by intracellular heme level (Chan *et al.*, 1993; Fukuda *et al.*, 1993a).

We have also found that intracellular heme is necessary for a continued increase in ferrochelatase mRNA (Fukuda *et al.*, 1993b). It is possible that a specific protein bound to ferrochelatase mRNA, which regulates the stability of mRNA, may be present in a similar fashion to that seen in the case of iron-regulated transferrin receptor mRNA (Klausner *et al.*, 1993). Thus, expression of ferrochelatase can be regulated at transcription and post-transcription in both erythroid and nonerythroid cells.

In an early study on the induction of ferrochelatase activity, ^{59}Fe-heme formation in MEL cells from exogenously supplied radioactive iron was finally increased 4 days following the addition of DMSO (Sassa, 1976). This method did not directly reflect enzyme activity. The increase in ^{59}Fe-heme synthesis was in disagreement with data resulting from direct measurement of ferrochelatase activity, since the enzyme activity began to increase within 12 h after the addition of DMSO (Nakahashi *et al.*, 1990a). On the other hand, transferrin receptor mRNA was dramatically induced between 48 and 72 h following DMSO treatment, and thereafter iron uptake by MEL cells increased during differentiation (Chan *et al.*, 1993). It is possible that the induction of transferrin receptors may be part of the erythroid differentiation program. Considering that multiple steps, which include iron uptake by cells and mitochondria as well as reduction of ferric ion, are involved in the incorporation of exogenous iron into heme, the iron supply may be a rate-limiting step of heme synthesis during erythroid differentiation. Although the MEL cells are a useful model for the elucidation of erythroid differentiation, other cells, including human

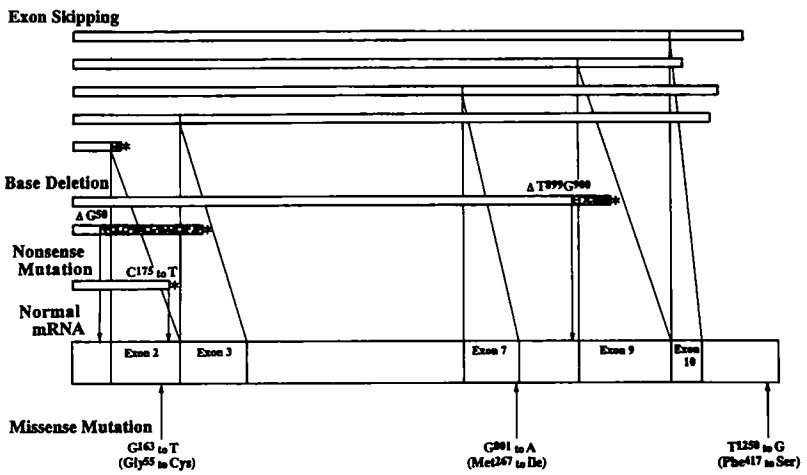

Fig. 1. Summary of genetic defects in EPP patients. Eleven types of genetic defects, five types of exon skipping, two types of base deletion, one nonsense mutation, and three types of missense mutation are summarized. Dotted line: abnormal amino acid sequence caused by frameshift; *: premature stop codon; Δ: base deletion.

erythropoietin-responsive cells (Sassa, 1980) and bone marrow cells (Sassa and Urabe, 1979; Beru and Goldwasser, 1985), exhibit a different regulatory mechanism by which erythropoiesis may be under the control of the PBG level. Therefore, multiple factors are concerned with the regulation of heme synthesis in erythroid cells.

FERROCHELATASE DEFECT IN HUMAN

Erythropoietic protoporphyria (EPP) is an inherited disorder of porphyrin metabolism, characterized clinically by photosensitivity and biochemically by a marked increase of protoporphyrin IX in erythrocytes, plasma, and feces (Magnus *et al.*, 1961). EPP, the most common form of erythropoietic porphyria in humans, is thought to be an autosomal dominant disease; however, the frequency of its clinical expression is highly variable (Kappas *et al.*, 1989). In patients with EPP, the activity of ferrochelatase is decreased to approximately 50% of normal levels in all tissues and isolated cell preparations so far examined: e.g., bone marrow cells (Bottomley *et al.*, 1975), liver (Bonkowsky *et al.*, 1975), cultured skin fibroblasts (Bloomer *et al.*, 1977), and lymphoblasts (Sassa *et al.*, 1982). Since the ratio between ferrochelatase activity and ALA synthase activity in the erythroid cells (approximately 2.6-times) is much lower than that in the liver (approximately 500 times), the phenotype of the heterozygote of the enzyme defect conforms to an erythropoietic-type porphyria. The deficiency of ferrochelatase activity results in the remarkable increase in protoporphyrin IX, a substrate for the enzyme, and accounts for cutaneous photosensitivity in this disorder.

Recently, detailed analysis of the molecular defects underlying EPP became possible, since cloning and sequence analysis of cDNA, as well as genomic DNA encoding human ferrochelatase, were reported (Nakahashi *et al.*, 1990b; Taketani *et al.*, 1992). Until today, molecular anomalies of ferochelatase from 11 patients have been demonstrated: 9 patients were autosomal dominant type while 2 were autosomal recessive type (Fig. 1). Of the 11 types of mutations identified, 5 (45%) were exon skippings, 3 (27%) were missense mutations, 2 were nucleotide(s) deletion, and 1 was a nonsense mutation. In all other types of porphyrias, including ALA dehydratase deficiency porphyria, acute intermittent porphyria, congenital erythropoietic porphyria, and porphyria cutanea tarda, 7 (18%) and 27 (69%) out of 39 reported mutations were exon skipping and missense mutations, respectively (Fujita and Nagai, 1993). Thus, exon skipping appears to be a common molecular mechanism underlying the ferrochelatase defect in EPP. It is also noted that mutated ferrochelatase genes in 8 out of 9 autosomal dominant cases encoded truncated proteins of ferrochelatase, whose activities should be essentially nil.

The most frequently observed skipping occurred in exon 10, though the way in which the exon was skipped was different in each case. In a patient with an autosomal dominant type of EPP, A1135 to T

substitution (-3 position of donor site of exon 10) resulted in exon 10 skipping (Wang *et al.*, 1993a), while in a patient with an autosomal recessive type of EPP, not only a to g substitution at +3 position of the exon 10 donor splice site but also T1088 to G substitution (6 bases upstream of the exon 10 acceptor splice site) were reported to cause defective splicing to exclude exon 10 from the mRNA (Sarkany *et al.*, 1994a). It should be noted that the nucleotide at 1088 bp in normal ferrochelatase cDNA is A, not T, and A1088 is 11 bases downstream, not 6 bases upstream, of the exon 10 acceptor splice site (Nakahashi *et al.*, 1990b; Taketani *et al.*, 1992). It is quite unusual that a mutation located 11 bases downstream of the exon 10 donor splice site results in exon 10 skipping. Ferrochelatase activity in white blood cells from the patient with autosomal dominant EPP decreased to 23% of the normal (Wang *et al.*, 1993a), whereas the enzyme activities in specimens from the patients with an autosomal recessive type EPP were 25% and 13% of mean control values (Sarkany *et al.*, 1994a). The difference in the decreased activity derived from the exon 10 skipping among these patients remains unclear, but it is possible that ferrochelatase activity in the patients varied depending on the intracellular level of heme since heme is necessary to maintain ferrochelatase mRNA (Fukuda *et al.*, 1993a,b). Screening of 21 patients from 19 unrelated families with EPP indicated two additional patients unrelated with A1135 to T substitution (Wang *et al.*, 1993b).

Ferrochelatases encoded in one allele of 4 patients have been revealed to lack either exon 2, 3, 7, or 9 (Nakahashi *et al.*, 1992; 1993a, b; Sarkany *et al.*, 1994b). The exon 2 skip resulted in a frameshift with a premature stop codon at 215 bp in the normal sequence (Nakahashi *et al.*, 1992). Thus, the aberrant mRNA would encode a truncated protein having only 29 amino acids. Since the existence of such a premature termination codon in a mRNA is known to significantly reduce its stability (Humphries *et al.*, 1984; Urlaub *et al.*, 1989; Kadowaki *et al.*, 1990), mRNA of ferrochelatase from the proband's cells decreased to approximately 50% of the normal control (Nakahashi *et al.*, 1992). None of the other exon skippings cause a frameshift; therefore, mutated ferrochelatase might be detected by the immunochemical analysis. However, ferrochelatase, either with exon 7 (encoding 33 amino acids) skipping or with exon 9 (encoding 55 amino acids) skipping, could not be detected by Western blot analysis. These aberrant proteins can be susceptible to proteolysis after their translations. Ferrochelatase activities of specimens from patients with exon 2, 7, or 9 skipping were 46.5%, 49%, or 42%, respectively, supporting the idea that these EPPs exhibit autosomal dominant traits. Many of these gene carriers had not developed any symptoms characteristic of EPP, although their ferrochelatase activities were decreased to almost 50% of the normal. Why some heterozygous carriers of the enzyme deficiency develop EPP, while others do not, remains an enigma, but this is also characteristic of other dominant forms of the porphyrias, such as acute intermittent porphyria, hereditary coproporphyria, and variegate porphyria (Kappas *et al.*, 1989).

In two patients with missense mutation of ferrochelatase so far reported, one was double heterozygous (Gly 55 to Cys in one allele and Met 267 to Ile in the other allele) whose enzyme activity decreased to 6% of the control (Lamoril *et al.*, 1991), and the other with a dominant form had Phe 417 to Ser substitution in the C-terminal end of the protein (Brenner *et al.*, 1992). Met 267 as well as Phe 417 are conserved among ferrochelatases from human, mouse and yeast (Nakahashi *et al.*, 1990b; Taketani *et al.*, 1990; Labbe-Bois, 1990). Expression of ferrochelatase either with Phe 417 to Ser or with Met 267 to Ile in *E. coli* indicated that the former had less than 2% of the normal activity while the latter showed increased thermolability (Dailey *et al.*, 1994). Marked decrease in the enzyme activity by the mutation of Phe 417 is very interesting, since mammalian ferrochelatase has an iron-sulfur cluster at the C-terminus of the protein (Dailey *et al.*, 1994; Ferreira *et al.*, 1994). The C-terminal truncated human ferrochelatase is inactive, and mutation of the Phe 417 of human enzyme diminishes the characteristic absorption spectra of the iron-sulfur cluster concomitant with a loss of the activity (Dailey *et al.*, 1994). Furthermore, disruption of the iron-sulfur cluster of human ferrochelatase by nitric oxide led to a loss of the activity, indicating that the iron-sulfur cluster would be essential for enzymatic activity (Furukawa *et al.*, 1995). Therefore, mutation at the C-terminal region would dramatically influence the structure and activity of the enzyme.

Wang *et al.*, (1993b) screened 21 EPP patients from 19 unrelated families, and showed that the mutations of G163 to A, G801 to A, g+1 to a at the donor splice site of exon 9, and T1250 to C were not found. These observations, coupled with two other patients with a nonsense mutation of G163 to T or with a two-base deletion (T899G900), resulted in ferrochelatase

activity reduction to 50% or to 40% of the control, respectively (Schneider-Yin *et al.*, 1994a); this indicates that EPP is a heterogeneous disease at the molecular level. Recently, a single base pair deletion (G40 del), which causes a frameshift resulting in a premature ochre stop codon at codon position 72 in exon 3, was identified in three out of six unrelated families from Northern Ireland (Todd *et al.*, 1993). This is the first report to suggest a common mutation causing EPP. Although the authors reported G40 deletion among these families, a figure of the automated DNA sequence in their manuscript seems to demonstrate G40 to C, C44 to G, and C48 to G mutations, which also abolishes the BssHII restriction site. Hence, further studies are needed for other evidence to identify a G40 deletion. Heterogeneous types of mutation have been described in human porphyrias, including EPP; this indicates that various mutations may be responsible for a single clinical disease entity. Further genetic studies on other EPP patients will answer the question if there are any common mutation(s) or not.

Three hundred cases of EPP were reported by 1976, whereas only 20 cases of EPP have been reported to develop hepatic failure by 1982 (Kappas *et al.*, 1989). Why some patients with EPP develop hepatic pathology while the majority do not is unknown. Sarkany *et al.* (1994a) have analyzed molecular defects of ferrochelatase from a family with the recessive form of EPP, whose ferrochelatase activities are decreased to 13–25% of the control, and suggested that inheritance of two disabling mutations in the ferrochelatase gene may be necessary for the development of liver failure (Sarkany *et al.*, 1994a). However, one double heterozygote and one putative double heterozygote of EPP, whose ferrochelatase activities were decreased by 94% and by 98%, respectively, had normal liver function (Lamoril *et al.*, 1991), indicating that the compound heterozygous cases are not always related to the liver failure (Schneider-Yin *et al.*, 1994b). Furthermore, two heterozygous patients with EPP, a Gin 58 to stop codon conversion and a exon 9 deletion, in both of which ferrochelatase activity decreased to 50% and 42% of the control, developed liver complication (Nakahashi *et al.*, 1993a; Schneider-Yin *et al.*, 1994a). These reports demonstrate that all of the EPP patients with liver failure are not always inherited in an autosomal recessive trait (Schneider-Yin *et al.*, 1994b). Further studies not only on the genetic analysis of mutation(s) but also on the environmental and acquired factors to affect liver disease seem to be necessary to elucidate the onset of hepatic failure in EPP.

FERROCHELATASE DEFECT IN HOUSE MOUSE

A chemical mutagenesis experiment of house mouse with ethylnitrosourea produced a viable autosomal recessive mutation causing jaundice and anemia (Tutois *et al.*, 1991). Homozygotes displayed hemolytic anemia, photosensitivity, cholestasis, and severe hepatic dysfunction. Protoporphyrin was found at high concentration in erythrocytes, serum, and liver. Ferrochelatase activity in various tissues was 2.7–6.3% of the normal control. Molecular analysis of the mouse ferrochelatase revealed Met 98 to Lys substitution (Taketani *et al.*, 1990; Boulechfar *et al.*, 1993). Expressed ferrochelatase with the mutation shows 6% of the activity of wild type. Heterozygotes have no signs and symptoms of EPP. Thus, mouse EPP is inherited in an autosomal recessive trait, in spite of the autosomal dominant trait of human cases. This difference in the pathogenesis has not yet been elucidated. One possible explanation is the difference in species between human and mice, since cattle protoporphyria, caused by deficiency of ferrochelatase, is also reported to be inherited in an autosomal recessive trait (Bloomer *et al.*, 1982). The other possible explanation is the difference of mutation between heterozygous cases and double heterozygous cases. In human, 8 out of 9 ferrochelatase proteins from the mutated allele in human heterozygous EPP are truncated; therefore, these enzyme activities should be nil. Another heterozygous case, Phe417 to Ser substitution, also decreased the enzyme activity to 1.5% of the normal control (Dailey *et al.*, 1994). Thus, it is concluded that the enzyme activity of all mutated ferrochelatases in human heterozygous EPP so far reported is essentially nil. The activity of the mutated mouse ferrochelatase (6% of control) seems to be much higher than those of the human heterozygous cases. It is well recognized that many gene carriers, whose ferrochelatase from one allele has no activity, have not developed any signs or symptoms of EPP. Therefore, the ferrochelatase activity (6% of control) from one allele may prevent the manifestation of EPP in the heterozygous mice. Although mice seem to provide a good model for the gene therapy of human EPP, mechanisms underlying the difference

of inheritance mode between human EPP and animal protoporphyria should be recognized.

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