expressed exclusively in erythroid cells. For hemoglobin synthesis in erythroid cells, the supply of protoporphyrin IX (PPIX) is regulated by ALAS2. ALA is then transported to the cytosol, and heme synthesis progresses successively in the cytosol following the second step. Coproporphyrinogen III (CoproIII), formed by uroporphyrinogen decarboxylase (UROD) at the fifth step, is imported into the mitochondria where the remaining three steps are performed. In the final step, reduced iron ($Fe^{2+}$) is inserted into PPIX by ferrochelatase (FECH), and then heme synthesis is completed.

Fe–S cluster is another major product that requires mitochondrial iron. As Fe–S cluster is an important electron transfer molecule, proteins containing Fe–S clusters are widely distributed and are involved in fundamental cellular activities, including multiple metabolic pathways, respiratory chain complexes, DNA synthesis, TCA cycle, and heme synthetic pathways.

When the utilization of mitochondrial iron is impaired by defects in heme synthesis, Fe–S cluster biogenesis, or disturbance of Fe–S cluster export, iron is accumulated in mitochondria. Excessively accumulated iron in mitochondria in erythroblasts becomes visible as iron granules by Prussian blue staining, and erythroblasts with perinuclear iron deposits are called ring sideroblasts. Such iron overload is likely to disturb cellular reduction–oxidation state and induce apoptosis, resulting in ineffective erythropoiesis. Anemia, which is characterized by this abnormal iron deposition in mitochondria in erythroblasts, is diagnosed as sideroblastic anemia. Reflecting the multiple mechanisms of disutilization of iron in mitochondria, the causes and clinical phenotypes of sideroblastic anemia are variable. This paper focuses mainly on the inherited form of sideroblastic anemia, and its pathophysiology is reviewed.

## 2 Inherited and acquired sideroblastic anemia

There are two forms of sideroblastic anemia, i.e., inherited and acquired sideroblastic anemia. Inherited sideroblastic anemia comprised heterogeneous phenotypes depending on the original function(s) of the mutated genes. To date, several genes responsible for inherited sideroblastic anemia have been identified. Responsible genes and phenotypes of each inherited sideroblastic anemia are summarized in Table 1. These genes play important roles in heme biosynthesis, Fe–S cluster biogenesis, or biology of mitochondria (Fig. 1). The incidence of inherited sideroblastic anemia is very rare, and the most frequent form is X-linked sideroblastic anemia (XLSA), which is caused by mutations in the erythroid-specific $\delta$-aminolevulinate synthase gene (*ALAS2*). Bergmann et al. [2] examined the gene mutations of 83 probands of inherited sideroblastic anemia, and identified mutations of *ALAS2*, *SLC25A38*, mitochondrial DNA (mtDNA), and *PUS1*, in 37, 15, 2.5, and 2.5% of cases, respectively. Disease-causing mutations were not found in the remaining 43% of cases, suggesting that there are as yet unidentified genes, mutations of which cause inherited sideroblastic anemia.

Compared to inherited sideroblastic anemia, acquired sideroblastic anemia is relatively common. Acquired sideroblastic anemia is caused by drugs or alcohol; however, the best-known acquired sideroblastic anemia is refractory anemia with ring sideroblasts (RARS), a subtype of myelodysplastic syndrome (MDS). As MDS comprised heterogeneous groups, unique genetic abnormalities in RARS have not been identified. However, genes in which mutations cause inherited sideroblastic anemia may also be involved in the development of RARS. For example, a chromosome anomaly, isodicentric X chromosome with breakpoints in Xq13-idic(X)(q13), has been reported to be

**Table 1** Inherited sideroblastic anemia

| | Inheritance | Chromosome | Gene | Mutation | Treatment | Affected organ |
|---|---|---|---|---|---|---|
| XLSA[a] | X-linked | Xp11.21 | ALAS2 | Missense | Vit B6 | – |
| XLSA/A[b] | X-linked | Xq13.1 | ABC7 | Missense | – | Nv |
| SA/GLRX5 | Autosomal recessive? | 14q32.13 | GLRX5 | Missense | ? | L |
| SA/SCL25A38 | Autosomal recessive? | 3p22.1 | SCL25A38 | Missense | ? | – |
| PMPS[c] | Maternal | Mitochondria | Mitochondria | Deletion | – | P, L, K, M, Nv |
| TRMA[d] | Autosomal recessive? | 1q23.3 | SCL19A2 | Missense | Thiamine | P, H, Nv |
| MLASA[e] | Autosomal recessive? | 12q24.33 | PUS1 | Missense | – | M |

*P* pancreas, *L* liver, *K* kidney, *M* muscle, *Nv* nerve system

[a] X-linked sideroblastic anemia

[b] X-linked sideroblastic anemia with ataxia

[c] Pearson Marrow Pancreas Syndrome

[d] Thiamine-responsive megaloblastic anemia

[e] Mitochondrial myopathy and sideroblastic anemia

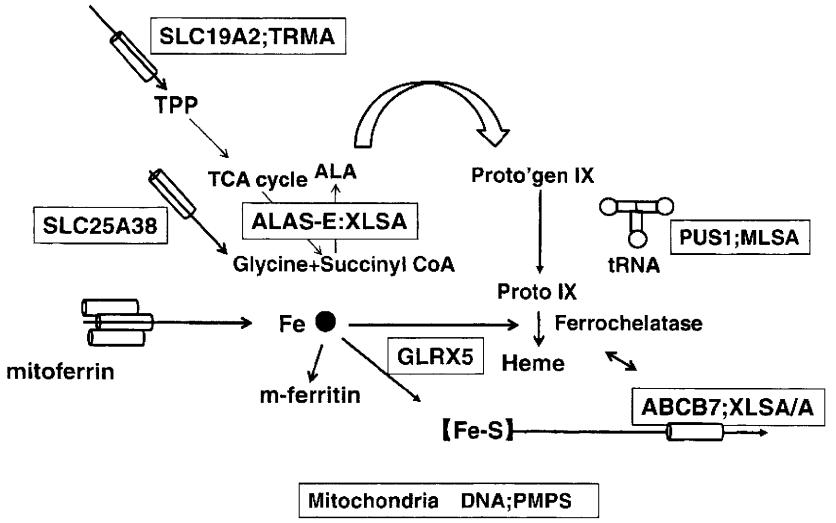


**Fig. 1** Genes mutated in inherited sideroblastic anemia are involved in mitochondrial iron homeostasis. ALAS2 is the initial enzyme of heme biosynthesis in erythroid cells. The deficiency results in impaired heme biosynthesis. SCL25A38 is a transporter of glycine, a substrate for ALA synthesis. SCL19A3 is a transporter for thiamine, which is a cofactor of α-ketoglutarate dehydrogenase involved in the synthesis of succinyl CoA, a substrate for ALA synthesis. ABCB7 functions in Fe–S cluster transport, whereas GLRX5 functions in Fe–S cluster biogenesis. Deletion of mitochondrial DNA and failure of uridine modification may lead to impaired mitochondrial function

associated with RARS. By cytogenetic analysis, the *ABCB7* gene, which is responsible for X-linked sideroblastic anemia with ataxia (XLSA/A), was shown to be located at the distal region (74.1 Mb) on Xq13. As the breakpoint of idic(X)(q13) clusters in the region proximal to the position of the *ABCB7* gene, the *ABCB7* gene should be hemizygously deleted in idic(X)(q13). It is, therefore, possible that loss of the *ABCB7* gene induces sideroblast formation in RARS with idic(X)(q13) by the same mechanism as that in XLSA/A. In addition, it was recently reported that the level of *ABCB7* gene expression in CD34+ cells in RARS is significantly decreased compared to other MDS subtypes and healthy subjects, and the percentage of sideroblasts is correlated with the level of *ABCB7* gene expression [3]. Although the mutation of the *ABCB7* gene is not detected, cultured erythroblasts from RARS patients exhibited low levels of *ABCB7* gene expression. These findings suggest that aberrant *ABCB7* gene expression may be involved in the development of RARS. In contrast, the level of *ALAS2* gene expression, which is most frequently mutated in inherited sideroblastic anemia, was reported to be increased. Consistent with this, PPIX was also found to be elevated in RARS. Together with the lack of *ALAS2* gene mutations in RARS, these findings suggest that aberrant expression or mutation of genes encoding components of the heme synthesis pathway is unlikely to be responsible for RARS. Besides *ABCB7* gene, mtDNA mutations are suspected to be related to MDS. Since large deletion of mtDNA causes Pearson Marrow Pancreas Syndrome (Pearson syndrome), which is an inherited sideroblastic anemia, it is possible that mutations of mtDNA may play a role in the development of RARS. Indeed, Wulfert et al. [4] found variety of mutations of mtDNA, including tRNA, cytochrome oxidase, ATPase, and cytochrome *b*, in MDS patients. Since mutations specific to RARS were not identified, it is unclear whether these mutations are related to sideroblast formation. However, it is possible that dysfunction of these genes may cause impaired mitochondrial metabolism, leading to disutilization of iron in mitochondria.

## 3 Gene mutation and clinical features of inherited sideroblastic anemia

### 3.1 X-linked sideroblastic anemia

The erythroid-specific δ-aminolevulinate synthase, ALAS2 (or ALAS-E), which requires pyridoxal 5′-phosphate (PLP) as a cofactor to exhibit its activity, is the first enzyme in the heme biosynthetic pathway in erythroid cells. It was reported that ALAS activity is decreased in the bone marrow of inherited sideroblastic anemia cases, suggesting that impaired heme biosynthesis may induce the onset of certain of sideroblastic anemias. It was then revealed that *ALAS2* is located at Xp11.21, and a mutation of *ALAS2* was first reported in a male patient with sideroblastic anemia in 1992 [5], confirming that mutations of *ALAS2* are responsible for the development of XLSA. Subsequently, *ALAS2* mutations were confirmed in familial cases originally reported by

Rundles and Falls; therefore, "Rundles and Falls syndrome" is now recognized as XLSA. ALAS2 deficiency is thought to result in a decreased supply of PPIX, which causes disutilization of iron leading to excessive accumulation of iron in mitochondria in erythroblasts. The characteristics of XLSA are as follows: (a) inherited in an X-linked fashion; (b) hypochromic and microcytic anemia; and (c) systemic iron overload. The most important feature of XLSA is that more than half of all patients respond to oral pyridoxine treatment. The conformation of ALAS2 protein is thought to be altered by missense mutations, resulting in decreased affinity of PLP, so that administration of a large amount of pyridoxine restores the enzymatic activity, which in turn leads to improvement of anemia. As of 22 April 2010, at least 48 mutations in 74 pedigrees have been identified, including our unreported cases (Table 2). The sites of mutations are distributed from exon 5 through exon 11, encompassing exon 9, which contains the lysine residue responsible for PLP binding. The results of pyridoxine treatment were reported for 49 of these 74 pedigrees. Complete or marginal pyridoxine responsiveness was reported in 39 pedigrees, while pyridoxine refractoriness was reported in 10 pedigrees. In unresponsive cases, the *ALAS2* gene mutations create premature stop codons, or may change the stability of the enzyme, leading to reduction of ALAS2 protein expression level. In addition to mutations in the coding sequences, mutations in the promoter region of the *ALAS2* gene were also identified in pyridoxine-refractory XLSA patients, suggesting that the disease may occur due to impaired transcriptional regulation of ALAS2 gene. Of note, it should be highlighted that the emergence of anemia in XLSA does not always occur during childhood. We reported a case with development of sideroblastic anemia at the age of 81 years while undergoing hemodialysis [6]. The diagnosis of XLSA was made based on a mutation in the fifth exon of the *ALAS2* gene, which decreased enzymatic activity of recombinant mutated ALAS2 protein. The clinical course of improvement of anemia by pyridoxine treatment supports the diagnosis of XLSA. The very late onset in this case of XLSA suggests that environmental stress, including nutritional deficiencies or, as in this case, hemodialysis therapy, may induce the emergence of disease in occult XLSA cases.

The establishment of an animal model of XLSA has been attempted by modification of *ALAS2* gene. *Alas2*-null mice died on embryonic day 11.5 due to severe anemia. *Alas2*-null mice lacked hemoglobinized erythroblasts, and large amounts of iron were accumulated in these erythroblasts [7]. However, iron accumulation was observed in the cytoplasm and typical sideroblasts were not seen. Definitive erythroblasts generated from *Alas2*-null ES cells in vitro were also not hemoglobinized, and again iron was accumulated in the cytoplasm, but not mitochondria, although biological changes in erythroblasts, including apoptosis and oxidative status induced by excess of iron, were observed [8]. This phenotypic discrepancy between ALAS2-deficient erythroblasts in human disease and those in the mouse model may be due to the species-specific background differences; however, ring sideroblast formation was observed in the fetal liver of *Alas2*-null mice partially rescued by the human *ALAS2* transgene. These results suggest that the formation of ring sideroblasts

**Table 2** Amino acid substitution of ALAS2 in patient with XLSA

| Ex. | Substitution | | No. of pedigree | | Ex. | Substitution | No. of pedigree | Ex. | Substitution | | No. of pedigree | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4 | L107P | | 1 | | 6 | R227C | 1 | 9 | R448Q | | 3 | |
| 5 | M154I | | 1 | | | S251P | 1 | | R452 | C | 6 (3) | 16 |
| | K156E | | 1 | | 7 | D263N | 1 | | | S | 2 | |
| | D159 | N | 1 | 2 | | C276W | 1 | | | H | 8 (2) | |
| | | Y | 1 | | | G291S | 1 | | R458H | | 1 | |
| | T161A | | 1 | | | K299Q | 1 | | I476N | | 1 | |
| | F165L | | 2 | | | V301A | 1 | 10 | Y506-fs | | 1 | |
| | R170 | S | 1 | 7 | 8 | D351R | 1 | | T508S | | 1 | |
| | | C | 2 (1) | | | T388S | 1 | | R517 | C | 1 | 2 |
| | | L | 3 (2) | | 9 | C395Y | 1 | | | G | 1 | |
| | | H | 1 | | | G398D | 1 | | P520L | | 2 | |
| | A172T | | 1 | | | R411C | 4 (1) | | H524D | | 1 | |
| | D190V | | 1 | | | G416D | 1 | 11 | R559H | | 1 | |
| | Y199H | | 1 | | | M426V | 1 | | R560H | | 3 | |
| | R204 | Q | 1 | | | R436W | 1 | | V562A | | 1 | |
| | | stop | 1 | | | | | | M567I | | 1 | |
| | | | | | | | | | S568G | | 2 (1) | |

As of 22 April 2010, at least 48 mutations in 74 pedigrees have been known. Our unreported cases were included, but promoter mutation was not included in this list

requires low levels of ALAS2, and complete loss of ALAS2 activity may shut off the iron transport into mitochondria. To clarify the mechanism of sideroblast formation and development of anemia in XLSA, the development of a viable mouse model is desirable.

### 3.2 Inherited sideroblastic anemia due to *GLRX5* mutation

Fe–S clusters function as active centers for electron transfer in enzymes involved in various cellular activities. Most proteins involved in Fe–S biogenesis are located in mitochondria in eukaryotes. Glutaredoxin 5 (GLRX5) is one of these proteins, and loss of grx5 function in yeast results in mitochondrial iron accumulation. In addition, GLRX5 is essential for Fe–S biogenesis in humans. The zebrafish mutant *shiraz* harboring GLRX5 deletion has severe anemia due to impaired heme synthesis caused by insufficient biogenesis of mitochondrial Fe–S clusters. The homozygous mutation of *GLRX5* was recently identified in an inherited sideroblastic anemia patient [9]. The mutation identified in the patient was shown to interfere with the splicing of intron-1, resulting in decreased *GLRX5* mRNA level. The patient presented with moderate anemia, hepatosplenomegaly, a reduced number of ring sideroblasts in the bone marrow, and iron overload.

Based on the analyses of zebrafish mutant *shiraz* and clinical data of the patient, the mechanism of sideroblast formation caused by *GLRX5* mutation was speculated as follows (Fig. 2). (1) Fe–S cluster biogenesis is decreased due to *GLRX5* mutation. (2) Decreased level of Fe–S clusters prevents the conversion of IRP1 (iron regulatory protein 1) to cytosolic aconitase, leading to increased level of IRP1 activity. (3) IRP1 represses translation of ALAS2 protein and stabilizes transferrin receptor mRNA through IRE. (4) Decreased level of ALAS2 results in deficiency of PPIX, whereas increased level of transferrin receptor results in stimulation of iron import. (5) Increased cellular iron level and disutilization of iron lead to iron deposition in mitochondria. (6) Deficiency of heme synthesis due to ALAS2 deficiency consequently stabilizes IRP2, resulting in repression of ALAS2 protein translation and stabilization of transferrin receptor mRNA. This successive loop through IRP for iron deposition suggests that the mechanism of sideroblast formation by GLRX5 deficiency is common with that of ALAS2-deficient activity.

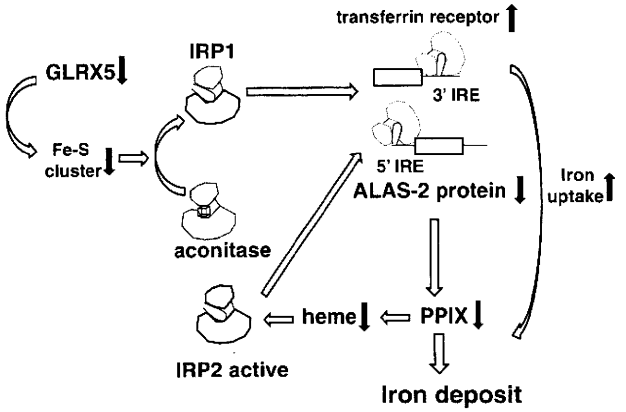


**Fig. 2** Mechanism of sideroblast formation due to GLRX5 deficiency. The mechanism of sideroblast formation by mutation of GLRX5 is speculated to involve heme deficiency and increased iron import by IRP activation, sharing that by mutation of ALAS2

### 3.3 X-linked sideroblastic anemia with ataxia

X-linked sideroblastic anemia with ataxia is a rare sideroblastic anemia inherited in an X-linked fashion similar to XLSA. XLSA/A patients exhibit early onset cerebellar ataxia, typically in the first year of life. The ataxia is usually stable or slowly progressive. The ataxia observed in XLSA/A patients may be related to the mitochondrial damage generated by iron loading in neural cells. The anemia of XLSA/A is hypochromic and microcytic. Anemia is typically mild, and is not responsive to pyridoxine. In addition, systemic iron overload is not observed. From the results of molecular analysis, mutation of the *ABCB7* gene, which is located at Xq13.1, was found to be responsible for the development of XLSA/A. To date, three missense mutations (Ile400 Met, Glu 433Lys, and Val 411Leu) of ABCB7 have been reported [10].

ABCB7 is a transporter protein, containing an ATP binding cassette, located in the inner membrane of mitochondria. In yeast deficient for ATM1p, which is a homolog of *ABCB7*, iron accumulates in mitochondria, and human ABCB7 rescues this phenotype. As ABCB7 is thought to be involved in export of Fe–S complexes, it is possible that impaired activity of ABCB7 affects the function of various molecules containing Fe–S clusters. Although the consequences of deficiencies of both GLRX5 and ABCB7 protein could be the same with regard to decreased cytosolic Fe–S clusters, the mechanism of iron accumulation in mitochondria induced by ABCB7 deficiency appears to be different from that by GLRX5, because erythrocyte protoporphyrin levels in XLSA/A patients were reported to be normal/high, suggesting normal ALAS2 function. It was shown that the interaction of ABCB7 with FECH increases the activity of FECH, which is the final enzyme in the heme synthetic pathway, and so it is possible that deficiency of ABCB7 affects the activity of FECH; however, FECH activity was normal in murine models. Thus, although iron may primarily accumulate and PPIX exists in mitochondria, accumulated iron is not available to FECH for heme synthesis in patients with

XLSA/A. The precise mechanism of mitochondrial iron accumulation in these patients remains to be elucidated.

### 3.4 Inherited sideroblastic anemia due to *SLC25A38* mutation

Recently, Guernsey [11] carried out SNP-based genome-wide scans in subjects with familial or sporadic congenital sideroblastic anemia, and identified several mutations of the *SLC25A38* gene. The pattern of mutation varies, including nonsense mutation, frameshifts, splice acceptor site mutation, and missense mutation, and inheritance is autosomal recessive.

SLC25A38 is a member of the inner mitochondrial membrane transporters, highly expressed in erythroid cells. The phenotypic abnormality of the yeast with deletion of the *SLC25A38* ortholog was consistent with the defect of heme biosynthesis, and the phenotype was rescued by supplementation with either glycine or 5-aminolevulinic acid (ALA), a substrate and product of ALAS, respectively. Therefore, it is hypothesized that SLC25A38 facilitates ALA production by transport of glycine into mitochondria. From the findings both in this study and recent analysis by Bergmann [2], the incidence of inherited sideroblastic anemia due to *SLC25A38* mutation appears to be high in inherited sideroblastic anemia. Therefore, it is necessary to examine the mutation in Japanese patients with sideroblastic anemia whose *ALAS2* mutation has not been identified or in cases of RARS.

### 3.5 Pearson Marrow Pancreas Syndrome (Pearson syndrome)

Pearson syndrome is a rare sideroblastic anemia accompanied by metabolic acidosis, ataxia and exocrine pancreatic insufficiency [12]. The disease is usually fatal, and patients die during infancy. Anemia is normomacrocytic, and neutropenia and thrombocytopenia are sometimes present. Pearson syndrome is caused by the deletion or duplication of mitochondrial DNA. The disease is usually sporadic, and occurs de novo. The mechanism of sideroblast formation in Pearson syndrome remains unclear. Deletion of mitochondrial DNA may result in deficiency of respiratory chain complex, including respiratory complex I (NADH dehydrogenase), complex IV (cytochrome *c* oxidase), and complex V (ATP synthase). Iron should be in the reduced state, "ferrous ($Fe^{2+}$) iron," when incorporated into PPIX by FECH in the final step of heme synthesis. It is speculated that cytochrome *c* oxidase functions to keep iron in the reduced state, and therefore the defect of cytochrome *c* oxidase fails to supply ferrous ($Fe^{2+}$) iron for FECH. Thus, iron is not utilized for heme synthesis, which results in mitochondrial iron overload.

### 3.6 Thiamine-responsive megaloblastic anemia

Thiamine-responsive megaloblastic anemia (TRMA) represents sideroblastic anemia with systemic symptoms, including insulin-dependent diabetes mellitus and neural hearing loss. TRMA is a rare autosomal recessive disorder, usually diagnosed during childhood. The anemia is macrocytic accompanied with megaloblastic features. The disease is responsive to thiamine supplementation, but not to folate, Vit B12, or pyridoxine. The gene responsible for TRMA is *SLC19A2*, which encodes a thiamine transporter [13]. It is not clear how mutations of *SLC19A2* are involved in sideroblast formation. As thiamine is required as a cofactor for α-ketoglutarate dehydrogenase, which is involved in the synthesis of succinyl CoA, a substrate for ALA, mutations of *SLC19A2* may induce sideroblasts by deficiency of heme. However, this speculation has yet to be confirmed; because there was a report that erythrocyte porphyrin level was not decreased in TRMA patients.

### 3.7 Mitochondrial myopathy and sideroblastic anemia

Mitochondrial myopathy and sideroblastic anemia (MLASA) is an extremely rare autosomal recessive disease characterized by myopathy, lactic acidosis, and sideroblastic anemia [14]. The disease is caused by molecular defects of the pseudouridylate synthase 1 gene (*PUS1*), which functions in pseudouridine modification of tRNAs [15]. Pseudouridine is an isomer of the nucleoside uridine, and is formed by enzymes called pseudouridine synthases. Pseudouridine is known to affect the structure of tRNA and to strengthen base pairing; therefore, failure of pseudouridine modification may lead to aberrant translation. There are not only cytoplasmic tRNAs but also mitochondrial tRNAs, and these mitochondrial tRNAs contain potential substrates for PUS1. Therefore, the mutation of *PUS1* may result in impaired biology of mitochondria.

## 4 Conclusion

Inherited sideroblastic anemia is a rare hematological disorder; however, it is very important to identify the genes, mutations of which are responsible for the disease, as analyzing their function will improve our knowledge of mitochondrial iron metabolism. Furthermore, if the mechanism of iron accumulation in mitochondria is common between inherited and acquired sideroblastic anemia, findings obtained in the study of inherited sideroblastic anemia could be helpful to understand the pathophysiology of acquired sideroblastic anemia, including RARS, and contribute to the establishment of specific treatment strategies.

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LETTER TO THE EDITOR

# The low expression allele (IVS3-48C) of the ferrochelatase gene leads to low enzyme activity associated with erythropoietic protoporphyria

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Erythropoietic protoporphyria (EPP) is an autosomal-dominant inherited disorder characterized biochemically by the excess accumulation and excretion of protoporphyrin, an intermediate precursor of heme biosynthesis. The enzyme abnormality that underlies protoporphyrin accumulation in EPP is a defect of ferrochelatase (FECH). Patients with EPP are clinically characterized by painful photosensitivity in skin and some (5–10%) exhibit liver failure due to massive hepatic accumulation of protoporphyrin [1, 2]. After we demonstrated the structure of the human *FECH* gene [3], more than 100 different kinds of molecular defects of FECH have been reported throughout the world. It has been reported that the low expression of a wild-type allelic variant *trans* to a mutated FECH allele is generally required for clinical expression of EPP [4]. According to this background, Gouya et al. [5] have found that the presence of a C at IVS3-48 in the human *FECH* gene causes the low expression of FECH. This intronic single nucleotide polymorphism (SNP) of the *FECH* gene, IVS3-48C/T transition, is key to the EPP phenotype. It is suggested that partially aberrant splicing of pre-mRNA by IVS3-48C is responsible for the clinical manifestations of EPP, although change in the enzyme activity has not been examined. Here, we report mutations of the *FECH* gene associated with IVS3-48C in five Japanese EPP patients. We found that the FECH activity of peripheral blood lymphocytes with IVS3-48C/C was <50% of that with IVS3-48T/T suggesting that the variations of the activity in patients with EPP could be based on the different levels of control.

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## 1 Mutation of the *FECH* gene in patients with EPP

We have diagnosed five patients with EPP in Japanese hospitals (Table 1). All patients suffered photosensitivity and three of them (patients 3, 4 and 5) developed hepatic dysfunction and died. Biochemical analysis of all patients showed marked elevation of protoporphyrin in erythrocytes. The FECH activity in peripheral blood lymphocytes of EPP patients decreased to 19–39% that of the control. After informed consent for all examinations had been obtained from patients and their families, blood samples were collected for genetic analysis. The total RNA was isolated by the guanidine thiocyanate method from lymphocytes or Epstein–Barr virus-transformed lymphoblastoid cells. cDNAs were synthesized with oligo(dT) primer using ReveTra Ace (Toyobo Co. Ltd., Tokyo, Japan). The entire FECH protein-coding region was amplified by PCR using two synthetic primers, 5′-GAGGCTGCCCAGGC A-3′ and 5′-TTTGCCTAACGCCACGGGGT-3′. The DNA fragments were ligated into pGEM-T vector (Promega Co., Madison, WI). Several plasmids-carrying FECH cDNA from a patient were isolated and the inserted DNAs were analyzed by sequencing. We found mutations in cDNAs. To confirm the mutation, we tried to analyze mutations of the *FECH* gene; namely, genomic DNA was isolated from whole blood cells. Regions containing molecular defects

**Table 1** Characterization of Japanese patients with EPP in terms of phenotype and genotype

| Patient no. | Sex | Age | Symptoms | Protoporphyrin in blood (μg/dl RBC) | Mutation in FECH | Genotype of normal allele IVS3-48 |
|---|---|---|---|---|---|---|
| 1 | M | 23 | Photosensitivity | 1,424 | IVS4(−4)a>g | C |
| 2 | M | 33 | Photosensitivity | 9,274 | Δ5b (751–755) | C |
| 3 | M | 41 | Photosensitivity liver failure | 12,574 | T557C (I186T) | C |
| 4 | M | 27 | Photosensitivity liver failure | 8,779 | Δ16b (574–589) | C |
| 5 | M | 36 | Photosensitivity liver failure | 9,127 | IVS9(+1)g>a | C |

found in FECH cDNA were amplified with primers as previously reported [6]. The amplified DNAs were directly sequenced. Then, we identified five different mutations that were the same as those previously reported for Japanese and European patients [2]. The common mutations between Asians and Caucasians can be ascribed to their common ancestry.

## 2 Relation of IVS3-48T/C of the *FECH* gene to Japanese EPP

The IVS3-48C/T transition of the *FECH* gene from EPP patients and their families was also analyzed. To amplify the DNA of the intron 3–exon 4 boundary (278 bp), the primers 5′-TCTACAACAAGAGAGCTGGC-3′ and 5′-ATCCTG CGGTACTGCTCTTG-3′ were used. Five Japanese EPP patients presented in this study were found to exhibit IVS3-48C of the normal allele (Table 1), which is consistent with the previous studies of Japanese [7], Caucasian and Asian EPP patients [2]. On the other hand, all carriers ($n = 4$) in their families were found with IVS3-48T of the normal allele. Other possible low expression alleles of the *FECH* gene, such as −251 G/A and IVS1-23C/T transitions linked to the disease [4], were also examined for the five EPP families, but the examination was not conclusive. Thus, the variation of IVS3-48C/T transition in the *FECH* gene may explain the difference in the residual enzyme activities in asymptomatic and symptomatic mutant carriers. Alternatively, because EPP development requires with the mutated allele of the *FECH* gene as well as the allele with IVS3-48C, it can be said that EPP is a recessive-inherited disease in a broad sense. We examined the relationship of decreased FECH activity with the genotype of the *FECH* gene, including IVS3-48C/T transition. After the isolation of peripheral blood lymphocytes of EPP patients and Japanese healthy controls, we examined the FECH activity by the formation of zinc-mesoporphyrin [8]; namely, homogenates from lymphocytes were incubated with mesoporphyrin (10 nmol), zinc acetate (40 nmol), Tween 20 (0.01%), and sodium palmitate (400 μg/mL) in 100 mM Tris–HCl, pH 8.0. The formation of Zn-mesoporphyrin was determined by

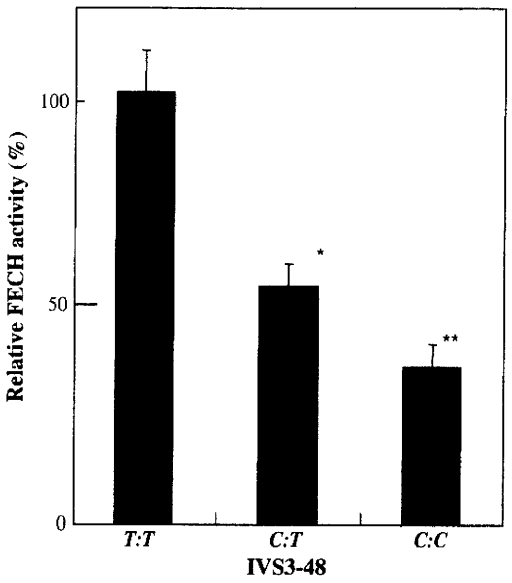


**Fig. 1** The FECH activity in peripheral blood T lymphocytes from healthy controls. Lymphocytes were isolated from healthy volunteers with IVS3-48T/T (*T:T*) ($n = 9$), IVS3-48C/T (*C:T*) ($n = 10$) and IVS3-48C/C (*C:C*) ($n = 4$) of the *FECH* gene. The FECH activity was measured using homogenates. The activity of 100% is equivalent to 67.2 ± 6.5 nmol Zn-mesoporphyrin formed/$10^6$ cells/h at 37°C with IVS3-48T/T. *$P < 0.01$, *C:T* versus *T:T*; **$P < 0.005$, *C:C* versus *T:T*

HPLC with 5C18-5AR column (4.6 × 150 mm) (Nacalai Tesque, Kyoto, Japan). As shown in Fig. 1, the highest activity was observed in the genotype with IVS3-48T/T, a moderate level was shown with IVS3-48C/T, and the lowest level was with IVS3-48C/C. The FECH activity with IVS3-48C/C was only 38% of that with IVS3-48T/T. Then, we compared the FECH activities in EPP patients with those in healthy controls with IVS3-48C/C, C/T and T/T. As shown in Fig. 2, the activities in EPP patients relative to those of the controls were divided into three groups, which corresponded to 15, 35 and 64% of the controls, and these were dependent on the three genotypes. Various investigators have found that the FECH activities in EPP patients vary widely (8–45%), compared with those in controls [1, 9]. Some researchers reported that EPP seemed to exhibit

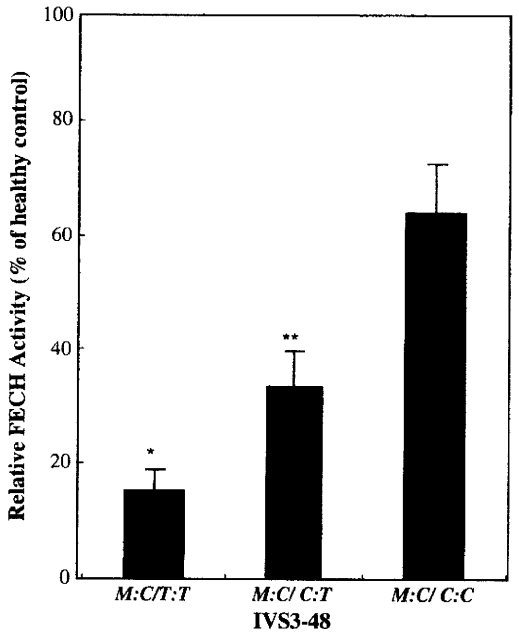


**Fig. 2** The FECH activity in EPP patients relative to that of healthy controls with IVS3-48C/T transition. The FECH activity in peripheral blood lymphocytes of patients with EPP (*M:C*) was measured by comparison with that of controls with IVS3-48T/T (*T:T*) ($n = 4$), IVS3-48C/T (*C:T*) ($n = 6$) and IVS3-48C/C (*C:C*) ($n = 5$) of the gene. * $P < 0.01$, *M:C/T:T* versus *M:C/C:C*; ** $P < 0.01$, *M:C/C:T* versus *M:C/C:C*

autosomal recessive inheritance owing to the low enzyme activity [10]. We now demonstrate that this variation is derived from the three different genotypes of the *FECH* gene. Thus, heterozygotes with the low expression allele (IVS3-48C) in combination with a null allele would produce a small amount of FECH when compared with the normal group. Similarly, a low expression allele combined with a missense allele could explain the weak FECH activity observed in patients with EPP. Conversely, the FECH activities in healthy controls varied, the level of the relative FECH activities in EPP patients differed, depending on the different activities from the IVS3-48 genotypes of the *FECH* gene among controls. To estimate the frequency of IVS3-48C/T transition of the *FECH* gene in the Japanese population, analysis by single-strand conformation polymorphism (SSCP) using GeneGel Excel 12, 5/24 kit (GE Bioscience, Buckinghamshire, UK) was carried out with the genomic DNA of healthy volunteers. Of the 148 Japanese examined, the genotype with IVS3-48C/C was found in 32 (22%), IVS3-48C/T was in 68 (46%) and IVS3-48T/T was in 48 (32%). Thus, over half of the subjects have IVS3-48C. This value is similar to those reported for Asian people [2, 7]. Given that 10% of Caucasians have IVS3-48C, Asian people including Japanese face a higher risk of EPP. Although the reduced FECH activity is an important factor to diagnose EPP, it is difficult to evaluate EPP by FECH activity because of the high frequency of healthy controls with IVS3-48C in Asian populations.

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# 鉄代謝の臨床　鉄欠乏と鉄過剰：診断と治療の進歩

## IV. 最近の話題
## 3. 鉄と炎症

張替　秀郎

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トピックス

# IV. 最近の話題
# 3. 鉄と炎症

張替　秀郎

要　旨

感染症などの炎症においては，ペプチドホルモンであるヘプシジンが分泌され，鉄の吸収・再利用が抑制される．また，細菌の鉄獲得を阻止するために様々な競合分子の発現が誘導される．この鉄動態の変化は，感染に対する防御機構として，また鉄の組織障害を回避する手段として有効であるが，長期間にわたる鉄利用サイクルの抑制は，慢性貧血の原因となる．

〔日内会誌　99：1282～1286，2010〕

**Key words**：鉄，炎症，感染症

## はじめに

鉄は，赤血球の酸素運搬に必須のヘモグロビン構成原子であるが，それ以外にも，ミトコンドリアの呼吸鎖，肝臓における薬物代謝，細胞増殖，DNA（deoxyribonucleic acid）合成など様々な生体反応に必須の原子である．一方で，鉄は活性酸素種を容易に産生する極めて毒性の高い原子でもある．この諸刃の剣である鉄を，有効にかつ安全に利用するために，生体は極めて巧妙な制御機構を有している．この鉄の巧妙な制御機構を理解する上で重要な点は，1．鉄が半閉鎖系で利用されていることと，2．さまざまな状況下でその動態がドラマティックに変化することである．本稿においては，この中で炎症に伴う鉄の生体内の動態とその制御機構について概説する．なお，本号においては本稿に関連する論文として，ヘプシジンおよび微生物の鉄捕捉分子についての総説が掲載されており，本稿と内容が重複する点があることをご容赦いただきたい．

はりがえ　ひでお：東北大学大学院医学系研究科血液・免疫病学分野

## 1．感染症と鉄代謝

生体内で炎症が起こる機序として，最も頻度が高く，また重要な機序は感染症である．特に細菌はその生存・増殖に鉄が必須であり，これまでに鉄過剰状態において，細菌感染症の増悪，遷延が起こることが数多く報告されている．例をあげると，*Yersinia*や*Vibrio*属の感染がヘモクロマトーシス患者において合併しやすいこと，また鉄過剰状態にあるサラセミア患者では，サルモネラ菌をはじめとする細胞内細菌の感染リスクが高いことが報告されている[1]．また，マウスモデルでは，ホロトランスフェリンの投与が*Nisseria meningiditis*感染を重症化させること，結核菌感染モデルにおいては鉄負荷が結核菌の増殖を著明に誘導することが報告されている[2]．生体内では自由鉄濃度は$10^{-24}$Mオーダーに低く抑え

**表．感染・炎症における鉄関連反応因子**

| 蛋白質 | 感染・炎症時における作用 |
|---|---|
| ラクトフェリン | 鉄の保持 |
| Nramp1 | ファゴソームからの鉄の排出 |
| リポカリン2 | ジデロフォアの捕捉 |
| ヘプシジン | 鉄の吸収・再利用の抑制 |
| IL-6，IL-1β | ヘプシジン誘導 |

られており，細菌が自由鉄にアクセスすることは困難であるため，細菌は特異的な分子を発現してトランスフェリン，フェリチンから鉄をキレートしたり，直接的にヘムを取り込むなどして，必要な鉄を獲得する．これに対する対抗措置として，生体は細菌への鉄の供給を抑えるために速やかに鉄の動態を変化させ，その結果，細菌感染から数時間以内に細胞外液，血清の鉄濃度が低下する．また，細菌感染時には細胞外だけでなく細胞内鉄動態にも変化が起こる．すなわち，マクロファージは自身に感染した細胞内細菌の増殖を阻害するために，細菌が寄生しているファゴソームから細胞質へと鉄を移動させることにより細菌を鉄欠乏状態へと導く．さらに，生体は細菌のキレート分子に対抗する競合分子を合成し，鉄濃度の減少だけでなく直接的に細菌の鉄の獲得を阻止するシステムを有している．これらの細菌の鉄の獲得を阻害する分子を表に示す．natural resistance-associated macrophage protein1（Nramp1）は好中球，マクロファージのファゴソームに発現する二価の金属イオンのトランスポーターである．Nramp1は鉄イオンを排出することにより，ファゴソーム内の鉄濃度を低下させ，ファゴゾームに寄生する細菌の生存・増殖を抑制すると考えられている．実際に，同遺伝子の変異により，結核菌，*Salmonella*菌，*Leishmania*菌などの細胞内寄生菌に対する易感染性が増すことが，マウス実験により確認されている[3]．なお，Nramp1のホモログであるNramp2は腸上皮細胞における食餌鉄の取り込み，および細胞内エンドソームからの鉄の排出に機能していることが知られている．ラクトフェリンはトランスフェリン類似の構造を持つ蛋白質であり，好中球などから分泌される．トランスフェリン同様に二つの三価鉄と強固に結合する．但し，トランスフェリンと異なり，感染巣や低酸素組織における酸性条件下で鉄を遊離しないため，細菌の鉄の獲得に競合的に働くものと考えられている．リポカリン2は主として好中球に含まれる糖蛋白質であり，細菌のジデロフォアであるエンテロバクチンに結合する．ジデロフォアは細菌が分泌するキレート分子であり，周囲の環境から鉄を獲得するために用いられる．リポカリン2はジデロフォアを捕捉することにより，細菌の鉄の利用を阻害し，その増殖を抑制する．リポカリン2は敗血症において大量に分泌されることが明らかとなっており，リポカリン2を欠損するマウスは敗血症が重症化しやすく，死亡率が高いことが報告されている[4]．このように，生体は細菌の増殖を阻止するために，可能な限り，細菌に利用される鉄を減らすための様々なメカニズムを用意している．

これらの防御反応は自然免疫に分類される生体反応であるが，獲得免疫にかかわる分子も鉄代謝において重要な機能を果たしている．古典的MHC（major histocompatibility complex）クラスI分子は，CD8陽性T細胞に抗原ペプチドを提示する分子であるが，β2マイクログロブリンと複合体を形成し，細胞表面上に発現している．MHCクラスI分子には，この古典的MHCクラスI分子の他に，β2マイクログロブリンと結合するものの，ペプチド結合性を持たない非古典的MHCクラスI分子が存在する．1994年にβ2マイクログロブリン欠損マウスが鉄過剰症をきたすことが見出され，MHCクラスI複合体の異常が鉄代謝異常の原因となることが明らかとなり[5]，その後，遺伝性ヘモクロマトーシス症例の解析から，非古典的MHCクラスI分子に属するHFE遺伝子がその責任遺伝子であることが明らかとなった[6]．HFE蛋白質のペプチド結合様構造は，その溝が

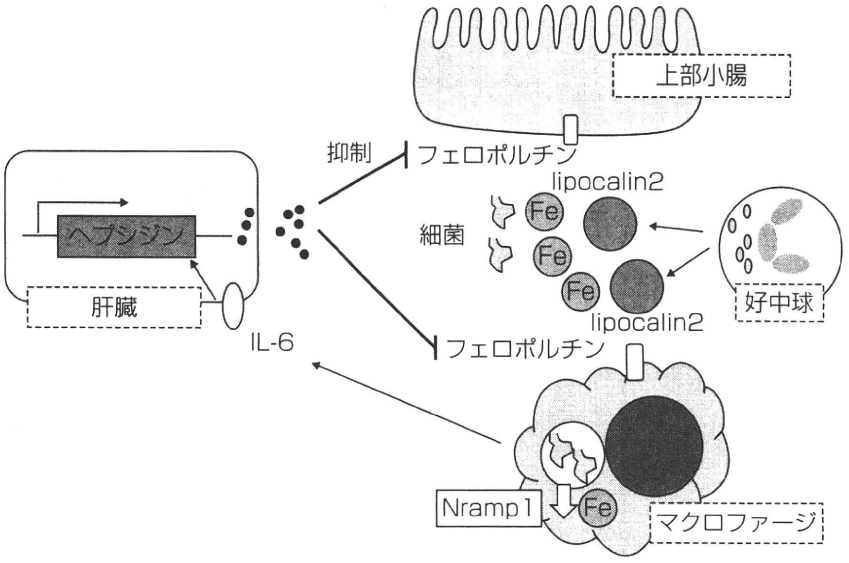



**図 1. 細菌感染時の鉄動態**

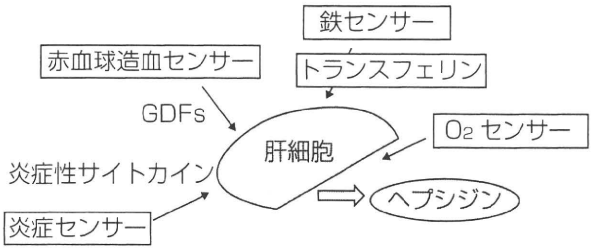
狭いため抗原提示ドメインとして機能しないが，β2マイクログロブリンと結合し複合体を形成する．遺伝性ヘモクロマトーシスにおいて最も頻度が高く認められる282番目のtyrosineがcysteinに変異したC282Y変異HFE蛋白質はβ2マイクログロブリンと結合できず，細胞表面に表出できないことが明らかになっている．*in vitro*の実験から，HFE蛋白質がトランスフェリン受容体1と結合することが明らかとなり，HFE蛋白質の変異による鉄過剰症は，HFEがトランスフェリン受容体1と結合できなくなるために，トランスフェリンとトランスフェリン受容体1の結合が促進することにより発症すると考えられていた．しかしながら，最近ではHFE変異による鉄過剰症の主因はヘプシジンの発現低下であると考えられている．すなわち，鉄が過剰になり，鉄で飽和されたホロトランスフェリンが増加すると，トランスフェリン受容体との結合能が低いHFEは受容体から遊離し，トランスフェリン受容体2と結合し同分子を安定化させる．トランスフェリン受容体2はBMP（bone morphogenetic protein）受容体コンプレックスと会合し，この受容体からのシグナルがヘプシジンの発現を誘導し，鉄の吸収，再利用が抑制される．一方で，HFE蛋白質に変異があると，トランスフェリン受容体2とHFEの会合が得られなくなるため，ヘプシジン誘導シグナルが細胞内に伝達されずヘプシジンの発現が低いままとなり，鉄が過剰となっても鉄利用サイクルの抑制がかからなくなるわけである．なお，ヘプシジンの具体的な鉄代謝制御機構については次々項で詳述する．鉄制御分子であるHFEがMHC分子の構造を有している生物学的合理性は明らかではないが，他の非古典的MHCクラスI分子も特異的機能を有していることが明らかとなっており，MHC分子の機能の多様性には何らかの発生学的な意義があるものと考えられる．ただし，HFE蛋白質は抗原提示能を有さないものの，マウスにおいてT細胞がHFE蛋白質をTCR（T-cell receptor）を介して認識すること，またミスフォールデインングされた変異HFE蛋白質がER（endoplasmic reticulum）に蓄積することにより，古典的MHCクラスI分子の細胞表面への移動が抑制されることも報告されている．これらの報告から示唆されるHFE蛋白質の免疫機能への関与は，免疫と鉄代謝が密接に関連していることを示すものである．

## 2. 非感染性炎症と鉄

感染性炎症時に細菌の鉄獲得を防ぐべく，鉄の利用サイクルが抑制されることは生体反応として理解がしやすいが，非感染性の炎症で同様の現象が誘導される生理的なメリットについては明らかとなっていない．ただし，関節リウマチ患者の滑膜液では，自由鉄濃度が高く，それに応じてラクトフェリン濃度が高くなっていることが報告されており，また，非感染性炎症時においてもフェリチン値が高値を示すことはよく知られた事象であることから，非感染性炎症時にも鉄の動態が変化し，鉄に対する防御機構が必要であることは理解できる．また，関節リウマチやSLE（全身性エリテマトーデス）においては鉄の負荷が，予後を悪くすることが報告されており，炎症における鉄の負の影響も明らかである[7]．炎症時には酸化ストレスレベルが通常に比べ極めて高くなること，炎症組織において過剰な鉄は酸化ストレスの産生要因となることを考えると，炎症時に自由鉄レベルを極力抑えることは組織保護のために合目的と考えられる．

## 3. 炎症における鉄の代謝機構

このように，生体内は，炎症時に鉄の再利用・吸収を抑え，いわば鉄利用サイクルを動かさない方向に鉄の動態をシフトさせる．この鉄動態のシフトの中心となる分子が，ヘプシジンである．ヘプシジンの鉄制御メカニズムを理解する上で，生体内の鉄の利用機構を把握することが重要であるため，まずそのシステムについて簡単に紹介する．鉄は半閉鎖系で利用されているため，体内の鉄の総量は3～4 gであるのに対し，十二指腸で吸収される鉄量は一日あたりわずか1～2 mgである．一方で，老化し処分される赤血球は一日当たり約20 mlの赤血球であり，この赤血球由来の鉄は約20 mgにのぼる．従って，赤血球造血に用いられる鉄のほとんどは分解された赤血球由来の再利用鉄ということになるが，食餌鉄，赤血球由来の再利用鉄，いずれも血清中のトランスフェリンに受け渡され，生体内鉄利用サイクルに入るにはフェロポルチンというトランスポーターを必要とする．すなわち，ヘム鉄，非ヘム鉄いずれの形であっても，上部小腸の上皮細胞から吸収された鉄はフェロポルチンによって血管腔側に放出され，トランスフェリンに受け渡される．また，マクロファージ内で分解された赤血球に由来する鉄は十二指腸細胞と同様にフェロポルチンを通じてマクロファージの外に排出されトランスフェリンに受け渡される．従って，フェロポルチンの発現レベルによって，生体内鉄利用サイクルの活動性が変化するといえる．このフェロポルチンの発現を制御する分子がヘプシジンである．ヘプシジンは当初肝臓に発現する抗菌ペプチドとして同定されたが，その欠損により著明な鉄沈着をきたすことから鉄代謝に関与している分子であると推測された[8]．その後，上部小腸での鉄吸収やマクロファージからの鉄排出に機能しているフェロポルチンがヘプシジンの受容体であり，フェロポルチンはヘプシジンと結合することによりエンドサイトーシスにより細胞内に取り込まれ分解されることが明らかとなった[9]．従って，実質的にヘプシジンはフェロポルチンの発現を抑制する分子であり，生体内の鉄利用サイクルを制御する中心的分子である．前述のように，鉄は生体にとって必須の原子であると同時に，極めて毒性の高い分子であるため，その必要量は生体内の状況に応じ，厳密にかつ迅速に変化する．感染，鉄の飽和状態，低酸素，赤血球造血など，鉄の利用量を増減するシグナルは多様であり，従ってヘプシジンは，これらのシグナルにより多層的な発現調節を受けている（図2）．この中で最も重要な点はIL-6（interleukin-6）などの炎症性サイトカインにより，肝臓でのヘプシジンの発現が誘導される点である．すなわち，感染




| | ヘプシジン | 鉄吸収・再利用 |
|---|---|---|
| Tf 飽和度↑ | ⇧ | ⇩ |
| GDF15 ↑ | ⇩ | ⇧ |
| IL-6 ↑ | ⇧ | ⇩ |
| O2 濃度 ↓ | ⇩ | ⇧ |

**図 2. ヘプシジン発現調節シグナル**
GDF : growth differentiation factor

症や慢性炎症によって分泌が促進するIL-6などの炎症性サイトカインは，ヘプシジンの分泌を亢進させ，結果として上部小腸，マクロファージにおけるフェロポルチンを介した鉄の吸収や放出が抑制させる[10]．このことにより，生体は細菌の鉄利用を阻止し，活性酸素種の発生による組織障害を回避する．これまで，慢性炎症により，鉄利用障害による貧血が誘導されることはよく知られていたが，この慢性炎症に伴う貧血の病態の中心がヘプシジンであることが明らかとなり，そのメカニズムが一元的に理解できるようになった．

## おわりに

炎症における鉄の動態とその分子機構について概説した．炎症時のヘプシジンによる鉄利用サイクルの抑制は細菌感染に対する防御や，鉄の組織毒性の防止において有効であることは理解できる．ただし，炎症が長期にわたり鉄の吸収・再利用の抑制が持続すると，鉄利用障害による，いわゆる慢性炎症に伴う貧血が発症し，生体にとって有益ではない．感染症，非感染性いずれの場合でも，原疾患の治癒による早期の炎症の鎮静化が基本であるが，今後この炎症に伴う貧血に対し，ヘプシジンを標的とした分子治療薬が開発されるものと考えられる．

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▷第42回東北支部総会　特別講演2◁

# 鉄代謝研究の進歩と鉄関連貧血

張　替　秀　郎

日　本　臨　床　検　査　医　学　会

▷第42回東北支部総会　特別講演2◁

# 鉄代謝研究の進歩と鉄関連貧血

張　替　秀　郎*

## Recent Progress in Iron Metabolism and Iron-related Anemia

*Hideo HARIGAE**

Iron is an essential metal not only in oxygen delivery, but also in cell proliferation and drug metabolism, while it is a very toxic metal producing reactive oxygen species (ROS). In order to avoid the toxicity and shortage of iron, the level of iron is strictly regulated in the body and cells. The central player regulating the amount of iron in the body is hepcidin. Hepcidin inhibits the release of iron from enterocytes and macrophages by accelerating the degradation of ferroportin, which is an exporter of iron. The amount of cellular iron is regulated by the IRE (iron responsive element) and IRP (iron regulatory protein) system. IRP1 and 2, whose activities depend on the concentration of cellular iron, bind to IRE, and regulate the translation of iron-related genes, which have IRE in 5' or 3'-UTR to balance iron uptake and utilization. Iron is utilized for the generation of heme and the iron-sulfur (Fe-S) cluster in mitochondoria. Mutations of genes involved in heme biosynthesis, iron-sulfur (Fe-S) cluster biogenesis, or Fe-S cluster transport cause an accumulation of iron in mitochondoria, leading to the onset of inherited sideroblastic anemia. The most common inherited sideroblastic anemia is X-linked sideroblastic anemia (XLSA) caused by mutations of the erythroid-specific $\delta$-aminolevulinate synthase gene (*ALAS2*), which is the first enzyme involved in heme biosynthesis in erythroid cells. However, there are still significant numbers of cases with genetically undefined, inherited sideroblastic anemia. Molecular analysis of these cases will contribute to the understanding of mitochondrial iron metabolism.

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**【Key Words】** anemia（貧血），red blood cell（赤血球），iron metabolism（鉄代謝），heme synthesis（ヘム合成），sideroblastic anemia（鉄芽球性貧血）

赤血球は含有する蛋白質の90%以上がヘモグロビンという酸素運搬にその機能を特化した細胞である。ヘモグロビンはヘムとグロビン蛋白質が4量体を形成した複合体であり，酸素分子はヘムの中心に配位された鉄に結合して運搬される。最近，鉄の生体内代謝機構が分子レベルで急速に解明されてきており，鉄関連貧血の病態が明らかとなってきた。本稿では，これらの制御機構を中心に最近の知見を紹介し，鉄関連貧血について概説する。

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## I. 生体内鉄代謝

鉄は，酸素運搬だけでなく細胞の分裂・増殖や呼吸などに必須の金属元素である。一方で，その反応性から極めて毒性の高い元素であるため，過剰にならないように吸収・代謝が厳密に制御されている[1]。体内の鉄の総量は3～4gであり，**Fig. 1**に示すように70%は赤血球に含まれるヘモグロビン鉄として利用されており，残りの30%がミオグロビン，呼吸酵素，代謝酵素の補欠分子としてのヘム鉄として利用されている他，網内系細胞に貯蔵されている。赤血球の寿命は120日であることから，毎日全体の1/120の赤血球，すなわち約25mlの赤血球が老化により処理されている。この処理された赤血球に含まれる25mgの鉄は新たな材料鉄として再利用される。食餌から吸収される外来性の鉄は一日1～2mgであるため，生体で利用される鉄は圧倒的に再利用鉄の方が多い。鉄利用をリサイクリングシステムに頼っているということは，鉄は容易に排出されないということを意味しており，輸血などで外部から強制的に鉄が入ると，生体は容易に鉄過剰状態になる。逆に，このシステムは体内の鉄のプールが減少した場合でも，簡単には鉄を外部から取り込むことができないということを意味しており，したがって出血が少量でも持続すると，生体は容易に鉄欠乏状態となる。例えば，生理的な出血が毎月60mlある女性の場合，月30mg，一日当たり1mgの鉄が失われていることになり，この量は通常の一日当たりの吸収鉄量に匹敵する。そのため，単純に考えると，この年代の女性は男性の2倍の鉄の摂取が必要であることになる。食餌鉄は主として十二指腸から吸収されるが，鉄の吸収形態として，非ヘム鉄とヘム鉄の二種類があり，吸収効率はヘム鉄が10～20%，肉以外に含まれる非ヘム鉄が1～5%とヘム鉄の方が吸収がよい[2]。吸収のメカニズムもヘム鉄と，非ヘム鉄とで異なっていることが，最近，明らかとなった。3価の非ヘム鉄は，腸管内腔側細胞膜に存在するduodenal cytochrome b(Dcytb)によって2価に還元され[3]，2価の金属トランスポーターであるdivalent metal transporter 1(DMT1)によって腸上皮細胞内に取り込まれる[4]。細胞内に取り込まれた鉄は血管内腔側細胞膜に存在するferroportinによって血管腔側に放出される[5]。一方ヘム鉄は腸管内腔側細胞膜に存在するheme carrier protein-1(HCP-1)によって細胞内に取り込まれ[6]，heme oxygenase-1(HO-1)によって分解され，非ヘム鉄同様ferroportinによって血管腔側に

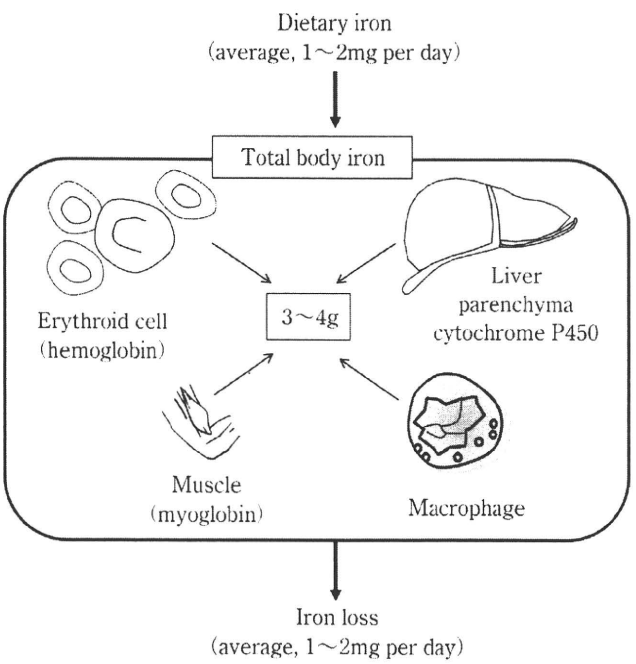


**Figure 1**　Distribution of iron in the body.
Seventy percent of the iron in the body is utilized in erythroid cells, while 10 to 15 percent is utilized in muscle and other tissues. Iron is stored in parenchymal cells of the liver and reticuloendothelial macrophages. The amount of iron in the body totals 3～4g, and 1 to 2mg of iron is absorbed and lost each day.

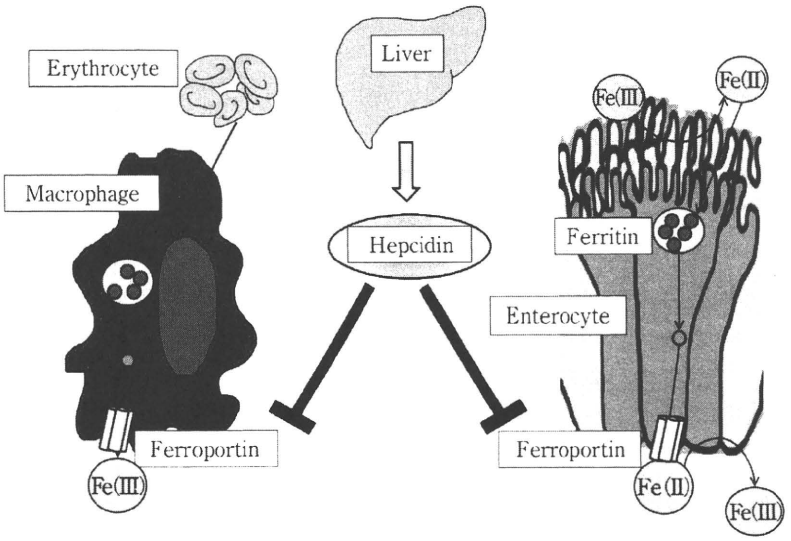


**Figure 2** Transport system of iron in the body.
Ferrous iron is transferred to plasma by ferroportin, the basolateral transporter, oxidized by hephaestin, and then carried by transferrin. Ferroportin also transfers iron from reticulo-endothelial macrophages to the plasma. Hepcidin suppresses ferroportin by facilitating the degradation of ferroportin.

放出される。排出された鉄は担送蛋白質であるトランスフェリンに結合するために，セルロプラスミン類似蛋白質である hephaestin により 2 価鉄から 3 価鉄へと変換される[7]。3 価鉄となりトランスフェリンと結合した鉄は骨髄に運搬され赤血球でのヘモグロビン合成に利用される。骨髄で産生された赤血球は，120 日間体内を循環した後，マクロファージに貪食され，細胞内で分解されたヘモグロビンから鉄が再利用される。この再利用鉄は十二指腸細胞と同じトランスポーターである ferroportin を通じてマクロファージから排出されるが，2 価鉄から 3 価鉄への変換はセルロプラスミンにより行われる。3 価鉄となった鉄はトランスフェリンに結合し再び骨髄へと運搬される。

さて，鉄を不足なく，また過剰にならないように，生体はどのようにこれらの鉄吸収・利用システムを調節しているのであろうか？ この鉄吸収・利用システムを中心的に調節している分子がペプチドホルモンであるヘプシジンである。ヘプシジンは当初肝臓に発現する抗菌ペプチドとして同定されたが，その欠損により著明な鉄沈着を来たすことから鉄代謝に関与している分子であると推測された[8]。その鉄代謝の調節は，腸上皮細胞やマクロファージからの鉄排出に機能している ferroportin を介したメカニズムによりなされていることが，既に明らかとなっている。すなわち，ヘプシジンは ferroportin と結合し，細胞内リソゾームへと誘導することにより，ferroportin を分解へと導く作用を有する[9]。ヘプシジンの作用により ferroportin の発現が腸上皮細胞やマクロファージで低下すれば，血液中へ鉄の排出が抑制され，鉄が蓄積されることになり，鉄利用が抑えられる（**Fig. 2**）。したがって，ヘプシジンは生体における鉄利用の抑制因子であるといえる。ヘプシジンの発現は多層的に制御されており，鉄の飽和度が高くなれば，ヘプシジンの転写は亢進し，同様に炎症により IL-6 などの炎症性サイトカインの濃度が高くなると，その転写が亢進する[10]。骨髄からの造血シグナルや，低酸素シグナルもヘプシジンの転写を変化させることが知られている。このように，生体はヘプシジンの発現を変化させることにより，需要状態に応じた鉄量の調節を行っているといえる。

## II. 細胞内鉄代謝

過剰鉄は細胞にとって活性酸素種を産生する危険元素であるため，鉄の代謝は細胞レベルでも精密に制御されていることが明らかとなっている。具体的には，細胞は鉄の吸収・貯蔵・利用に関わる遺伝子の発現を鉄濃度によって協調的に調節し，生体同様過不足なく鉄量を調節するメカニズムを兼ね備えている。それが IRE (iron responsive element) を介した IRP (iron regulatory protein) による発現制御システムである[11]。IRE は mRNA の非翻訳領域に存在す

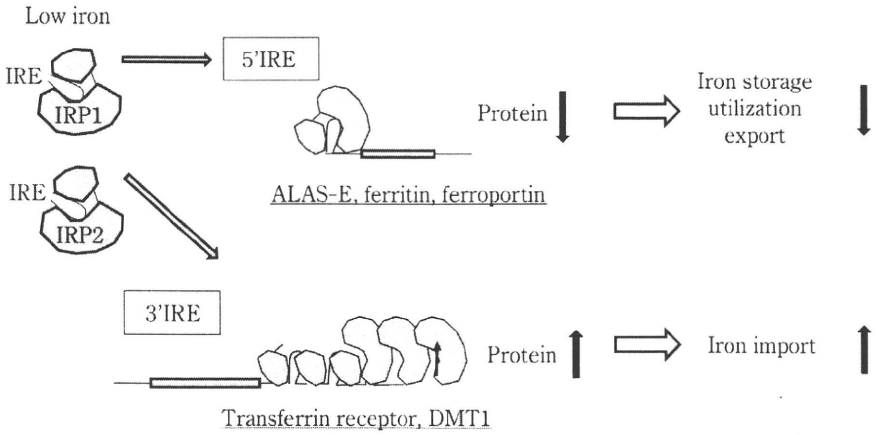


**Figure 3**　The expression of iron-related genes is regulated by the iron regulatory protein (IRP) and iron responsive element (IRE) systems.

In a low iron environment, IRP1 and 2 bind to the iron-responsive element (IRE). When bound to the 5'UTR IRE, they inhibit the translation of *ALAS2*, *ferritin*, and *ferroportin* genes. On the other hand, they increase the mRNA stability of the *Transferrin receptor*, the *DMT1* gene, when bound to the 3'UTR IRE.

る高次構造で，"CAGUGN(N は G 以外の塩基)" の塩基配列がループ構造をとり，その両端の 10～12 塩基が相補となり 2 本鎖を形成する。この IRE に IRP が結合し，制御対象となる鉄関連遺伝子の翻訳を調節している。重要な点は，IRE が 5'UTR に存在する場合と 3'UTR に存在する場合でその作用が逆という点である(**Fig. 3**)。すなわち，IRE が 5'UTR に存在する場合は，IRP の IRE への結合により翻訳が抑制されるが，3'UTR に IRE が存在する場合は，IRP の結合により mRNA の安定性が増すため，翻訳が亢進する[11]。IRP には IRP1 と IRP2 の 2 種類があり，IRP1 は細胞内鉄濃度が高い場合は TCA サイクルの aconitase として機能しているが，鉄が低濃度になると IRP として機能する。一方，IRP2 は，鉄が高濃度の場合はユビキチン化によりプロテオソームで分解されるが，低濃度では分解されずに IRP として機能する[12]。5'UTR に IRE が存在する遺伝子は，赤血球におけるヘム合成系の初発酵素である *ALAS2*，フェリチン，ferroportin，3'UTR に IRE が存在する遺伝子は DMT1，トランスフェリンレセプターなどである。細胞内鉄濃度が高い場合は IRP が機能しないため，ALAS2，フェリチン，ferroportin の発現が亢進する一方，DMT1，トランスフェリンレセプターの発現が低下する。すなわち，鉄の貯蔵，利用，排出が高まり，取り込みが低下することになる。逆に，細胞内鉄濃度が低い場合は IRP が機能するため，ALAS2，フェリチン，ferroportin の発現が低下する一方，DMT1，トランスフェリンレセプターの発現が亢進する。すなわち，鉄の貯蔵，利用，排出が低下し，取り込みが高まることになる(**Fig. 3**)。

### III. ミトコンドリア内鉄代謝

トランスフェリンに結合した鉄はトランスフェリンとともにトランスフェリンレセプターと結合し細胞内に取り込まれた後，pH の低いエンドソームで結合が解かれる[13]。前述の腸上皮細胞のトランスポーターである DMT1 はエンドソームにおいても発現しており，鉄は同分子を介してエンドソーム外に排出される。排出後，ミトコンドリアまでの鉄の輸送経路に関しては未だ明らかではない。ミトコンドリアに到達した鉄は，ミトコンドリア内膜に存在するトランスポーターであるミトフェリンを介してミトコンドリアに取り込まれる。ミトコンドリアに移行後，鉄はヘム合成，鉄-硫黄クラスター合成に利用される。ヘム合成を触媒する酵素は 8 種類からなり，最初の酵素と最後の 3 つの酵素はミトコンドリア内に存在し，中間の 4 つの酵素は細胞質に存在する[14]。赤血球における最初のステップは赤血球型 5-アミノレブリン酸合成酵素(ALAS-E; *ALAS-2*)による 5-アミノレブリン酸の合成である。その後，順次ポルフィリン環が合成され，最終的に出来上がったポルフィリン環にフェロケラターゼにより鉄が挿入され，ヘムが完成する(**Fig. 4**)。ヘムはミトコンドリアから排出され，グロビンと結合しヘモグロビンとなる