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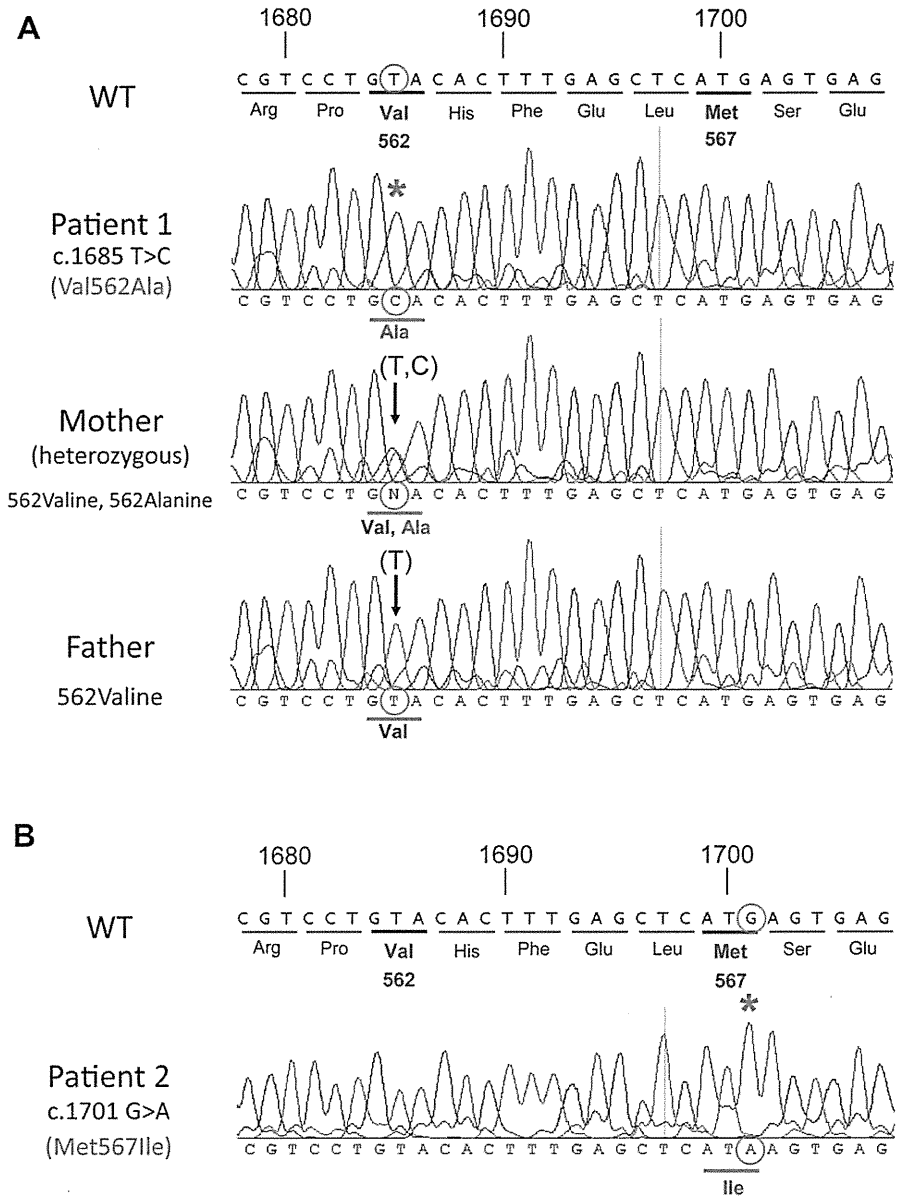
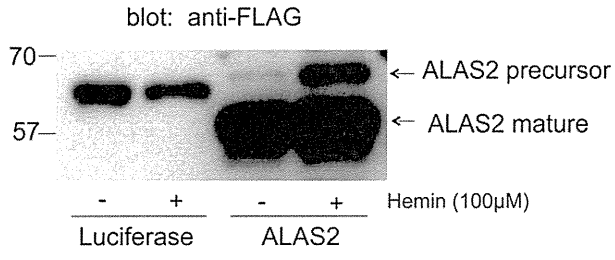


Supplementary Figure E1. Direct sequencing of 11th exon of ALAS2 gene in patients with sideroblastic anemia. Exon 11 of ALAS2 gene from each proband was amplified by PCR, and the amplicon was sequenced directly. Numbers shown at top indicate the positions of cDNA sequence, which is started from the first nucleotide of the ATG-translation initiation codon. Second and third lines indicate wild-type DNA sequence and amino acid sequence, respectively. Identified mutations are indicated with asterisks, and the expected amino acid substitution is shown under each mutation. (**A**) The c.1685T>C mutation of ALAS2 gene in case 1. The heterozygous condition of proband's mother and the wild-type allele of proband's father are shown. (**B**) The c.1701G>A mutation of ALAS2 gene in case 2.



Supplementary Figure E2. Transient expression of FLAG-ALAS2 and FLAG-Luciferase in HeLa cells. HeLa human cervical cancer cells were transfected with FLAG-ALAS2 or FLAG-luciferase expression vector, then treated with 100 μM hemin. Cell lysates were subjected to the Western blot analysis with anti-FLAG antibody. Shown are the representative data.

Ann Hematol (2013) 92:1–9
DOI 10.1007/s00277-012-1564-5

ORIGINAL ARTICLE

Clinical and genetic characteristics of congenital sideroblastic anemia: comparison with myelodysplastic syndrome with ring sideroblast (MDS-RS)

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Received: 30 May 2012 / Accepted: 21 August 2012 / Published online: 16 September 2012

Abstract Sideroblastic anemia is characterized by anemia with the emergence of ring sideroblasts in the bone marrow. There are two forms of sideroblastic anemia, i.e., congenital sideroblastic anemia (CSA) and acquired sideroblastic anemia. In order to clarify the pathophysiology of sideroblastic anemia, a nationwide survey consisting of clinical and molecular genetic analysis was performed in Japan. As of January 31, 2012, data of 137 cases of sideroblastic anemia, including 72 cases of myelodysplastic syndrome (MDS)–refractory cytopenia with multilineage dysplasia (RCMD), 47 cases of MDS–refractory anemia with ring sideroblasts (RARS), and 18 cases of CSA, have been collected. Hemoglobin and MCV level in CSA are significantly lower than those of MDS, whereas serum iron level in CSA is significantly higher than those of MDS. Of 14 CSA for which DNA was available for genetic analysis, 10 cases were diagnosed as X-linked sideroblastic anemia due to *ALAS2* gene mutation. The mutation of *SF3B1* gene, which was frequently mutated in MDS-RS, was not detected in CSA patients. Together with the difference of clinical data, it is

Electronic supplementary material The online version of this article (doi:10.1007/s00277-012-1564-5) contains supplementary material, which is available to authorized users.

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suggested that genetic background, which is responsible for the development of CSA, is different from that of MDS-RS.

Keywords Congenital sideroblastic anemia · Myelodysplastic syndrome · ALAS2

Introduction

Sideroblastic anemia is characterized by anemia with the emergence of ring sideroblasts in the bone marrow. Ring sideroblasts are formed by the irregular accumulation of iron in mitochondria. There are two forms of sideroblastic anemia i.e., congenital sideroblastic anemia (CSA) and acquired sideroblastic anemia. Most of acquired sideroblastic anemia cases were included in myelodysplastic syndrome (MDS). To date, mutations of genes involved in heme biosynthesis, Fe–S cluster biogenesis, or the biology of mitochondria have been reported in CSA [1–5]. Impaired function of these genes is speculated to result in disutilization of iron, leading to accumulation of iron in mitochondria. Acquired sideroblastic anemia in MDS is categorized either as refractory cytopenia with multilineage dysplasia (RCMD) or refractory anemia with ring sideroblasts (RARS) depending on the level of dysplasia. In contrast CSA, mechanism of forming ring sideroblasts in MDS is not clarified, although it was recently suggested that the mutations of splicing pathway are involved in the pathogenesis of MDS [6]. It is possible that there is a common mechanism between CSA and MDS; however, mutations in genes, which are responsible for development of the CSA, have not been identified in MDS.

The most common form of CSA is X-linked sideroblastic anemia (XLSA), which is caused by mutation of erythroid-specific 5-aminolevulinate synthase (*ALAS2*), the first enzyme of heme synthesis in erythroid cells [7–10]. More than half of the patients with XLSA respond to the administration of pyridoxine [vitamin B6 (Vit.B6)], or pyridoxal 5-phosphate (PLP), which is the coenzyme of *ALAS2* [11]. In XLSA, adult onset cases have been reported [12, 13]; therefore, it is possible that some cases of CSA may be misdiagnosed as MDS, especially RARS. However, the clinical and pathological features of congenital and acquired sideroblastic anemia have not been fully clarified because there have been no comprehensive studies, including clinical and genetic analyses, focusing on sideroblastic anemia.

Here, we performed a nationwide survey of sideroblastic anemia in Japan to investigate the epidemiology and pathogenesis of this disease. The difference of clinical data and results of genetic analysis suggest that genetic background, which is responsible for the development of CSA, is distinct from that of MDS-RS.

Materials and methods

Data acquisition

This study consisted of three investigations. First, patients with sideroblastic anemia were searched by questionnaire sent to hospitals with hematology department (493 hospitals) and pediatric hematology department (593 hospitals) asking for information about patients diagnosed as sideroblastic anemia (first investigation) over the past 10 years. Next, detailed clinical data of sideroblastic anemia patients were collected from the hospital based on responses to the first investigation (second investigation). Survey items were age of onset, gender, family history, hematological and biochemical findings, treatment, and cause of death. Then, genetic analysis of patients, who were diagnosed as CSA and MDS without chromosomal anomaly, was performed in cases for which genome sample was available (third investigation).

This study was approved by the ethics committee of Tohoku University Graduate School of Medicine, the center responsible for clinical and genetic analysis. Informed consent for the genetic analysis was obtained in all cases.

Diagnostic procedure

Ring sideroblasts were defined following the 2001 World Health Organization (WHO) classification. Sideroblastic anemia patients were diagnosed in the respective institutions. In all cases, bone marrow smears were investigated, and at least 15 % ring sideroblasts were confirmed by iron staining. Furthermore, diagnosis for RARS was made when dysplasia restricted to erythroid lineage in bone marrow was recognized. Diagnosis for RCMD was made when there is multilineage dysplasia. Thereafter, in the present study, RCMD correspond to refractory cytopenia with multilineage dysplasia and ringed sideroblasts (RCMD-RS) of the 2001 WHO classification. Diagnosis for CSA was made when the patient had a family history or the disease onset during infancy, or fulfilled the characteristic features of XLSA, such as onset at a young age, microcytic anemia, and responsiveness to Vit.B6.

Genetic analysis of patients with sideroblastic anemia

In the genetic analysis, mutations in *ALAS2*, *SLC25A38*, *GLRX5*, *ABCB7*, *PUS1*, and *SLC19A2,* which are known to be responsible for CSA, were examined in 14 cases of CSA and 10 cases of MDS. In addition, *SF3B1*, which was very recently reported to be mutated in sideroblastic anemia in MDS at a high incidence, were analyzed as well. Mutation analysis for the *ALAS2* gene was performed first in all candidates, and then the analysis proceeded to the other

genes if no mutations in *ALAS2* were detected. For mutation analysis of *ALAS2*, genomic DNA was extracted from the proband's peripheral blood using QIAamp DNA blood midi kit (QIAGEN, Valencia, CA, USA). The proximal promoter region [14], erythroid enhancer in intron 8 [15], and all exons and exon–intron boundaries of the *ALAS2* gene were amplified using ExTaq DNA polymerase (Takara Bio, Shiga, Japan) [16]. Amplified products were purified using a QIAquick gel extraction kit (QIAGEN) after agarose gel electrophoresis. They were then subjected to direct sequencing analysis using BigDye Terminator Cycle sequencing kit v1.1 with an ABI3100 genetic analyzer (Life Technologies Corp., Carlsbad, CA, USA). Mutation of the gene was confirmed by repeated polymerase chain reaction (PCR) followed by direct sequencing analysis. Genes other than *ALAS2* were sequenced by Hiseq2000® [6]. Briefly, genomic DNA was amplified using REPLI-g mini kit® (QIAGEN Science). After adjusting the concentration of amplified DNA, DNA from consecutive 12 samples was combined into one DNA pool, and the entire coding sequences were amplified by primers to which *Not*I linker was attached. The products were digested with *Not*I, and ligated with T4 ligase. Then, DNA was sonicated into ~200-bp fragments, and sequencing libraries were generated. Libraries were subjected to deep sequencing on Hiseq2000®. Sequencing data was analyzes as described previously. Detected mutations were validated by direct sequence.

Analysis of enzymatic activity of recombinant ALAS2 protein

For preparing recombinant ALAS2 proteins, complementary DNA (cDNA) encoding mature human ALAS2 protein was amplified using a following primer set (5′-GGTGGTCATATGATCCACCTTAAGGCAACAAAGG-3′and 5′-GGCATAGGTGGTGACATACTG-3′). The amplified cDNA was then treated with *Nde*I restriction enzyme and was cloned between *Nde*I and blunt-ended *Sap*I site of pTXB1 plasmid (New England Biolabs, Ipswich, MA, USA), resulting in pTXB-AEm. From this plasmid, mature ALAS2 protein was expressed as an inducible fusion protein with Intein and chitin-binding domain in *E. coli*. To obtain the mutant protein, the identified mutation was introduced into pTXB-AEm using PrimeStar Max site-directed mutagenesis kit (Takara Bio, Shiga, Japan). For expression and purification of wild-type and mutant ALAS2 proteins, *E. coli* BL21 (DE3) was transformed with each plasmid. The induction and purification of the recombinant proteins were performed using Impact system (New England Biolabs) according to manufacturer's instruction. Briefly, each recombinant protein was induced in *E. coli* with 0.1 mM IPTG at 25 °C for overnight. Then, cells were resuspended with lysis buffer (20 mM Tris–HCl pH8.5, 500 mM NaCl, 1 mM EDTA, 0.1 % Triton X-100, 1 mM PMSF, 1 μg/ml of antipain, pepstatin, and leupeptin). After the sonication and centrifugation, cleared cell lysates were incubated with chitin beads for 1 h at 4 °C, then washed with wash buffer (20 mM Tris–HCl pH8.5, 500 mM NaCl, 1 mM EDTA, and 0.1 % Triton X-100). Tag-free recombinant mature ALAS2 protein was obtained by on-column cleavage with 50 mM DTT in wash buffer at room temperature for 16 h. After the elution from the column, protein concentration was determined using Bio-Rad Protein assay reagent (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The ALAS activity of each recombinant protein was measured in vitro, as described previously [8].

Statistical analysis

Results are presented as means±SD with the exception of the age of onset, which is expressed as the median. Statistical analysis was performed using Student's *t* test, and $P<0.05$ was taken to indicate statistical significance.

Results

Epidemiology of sideroblastic anemia

As of 31 January 2012, detailed data for 148 sideroblastic anemia, including MDS and secondary sideroblastic anemia, patients have been collected. Excluding 10 cases of refractory anemia with excess blasts (RAEB) and one case of sideroblastic anemia due to alcohol, the remaining 137 cases were classified as 18 cases of CSA, 47 cases of RARS, and 72 cases of RCMD. Of 18 CSA cases, 7 were already confirmed to be XLSA due to mutation of *ALAS2* before registration in this study, and the others were diagnosed as CSA based on family history or clinical findings, including responsiveness to Vit.B6 treatment. Clinical findings and family history, which suggest the porphyria, were not observed in any CSA patients.

Analysis of the pathology of congenital sideroblastic anemia

Laboratory data of CSA, RARS, and RCMD are shown in Tables 1 and 2. Median age at onset of CSA was younger than those of RARS and RCMD (19, 72.5, and 71 years old, respectively). Hemoglobin and mean corpuscular volume (MCV) values of CSA were significantly lower than those of RARS and RCMD cases (7.1 g/dl and 69.0 fl, 8.7 g/dl and 106.8 fl; and 8.3 g/dl and 106.5 fl, respectively). Serum iron level in CSA was significantly higher than that in RARS or RCMD (210.7, 162.8, and 171.1 μg/dl, respectively). These data have possibilities of reflecting the states of the iron over-loaded of CSA; however, as serum iron concentration is very instable and depends from different factors, this finding should be carefully evaluated.

Table 1 Clinical data of CSA, RARS, and RCMD (1)

	CSA (n=18)	RARS (n=47)	RCMD (n=72)	p-value (between CSA and RARS)	p-value (between CSA and RCMD)
Gender					
Male	17	33	44		
Female	1	14	28		
Median age at onset (year)	19.0 (±20.2)	72.5 (±10.4)	71.0 (±13.0)	<0.01	<0.01
White blood cells (/μl)	5547 (±2022)	4741 (±2561)	4105 (±1847)	0.24	<0.01
Red blood cells ($\times 10^4$/μl)	383.4 (±100.0)	245.6 (±45.6)	239.4 (±56.4)	<0.01	<0.01
Hemoglobin (g/dl)	7.1 (±2.21)	8.7 (±1.7)	8.3 (±1.8)	<0.01	0.02
Mean corpuscular volume (fl)	69.0 (±11.6)	106.8 (±9.0)	106.5 (±9.2)	<0.01	<0.01
Platelet ($\times 10^4$/μl)	28.5 (±12.62)	25.9 (±15.5)	23.9 (±24.1)	0.53	0.44
Reticulocyte (‰)	12.1 (±10.9)	17.7 (±10.8)	21.5 (±20.1)	0.07	0.05

When iron-related laboratory data were examined in transfusion independent cases (CSA, 13; RARS, 26; RCMD, 34), Serum iron level in CSA was tended to be higher than that in RARS or RCMD (210.6, 180.3, and 166.6 μg/dl, respectively), although the difference was not significant (p=0.07, data not shown). Serum ferritin level in CSA, RARS and RCMD were elevated in these transfusion independent cases (1,087.9, 898.1, and 732.2 ng/ml, respectively), suggesting that most of sideroblastic anemia patients were iron-overloaded before transfusion. There were no significant differences in other biochemical data among the three groups.

Chromosomal abnormalities of MDS

Data regarding cytogenetic abnormalities were available for all RARS patients and for 68 of 72 RCMD patients. Figure 1 shows the cytogenetic findings of RARS and RCMD. In RARS cases, chromosomal abnormalities were found in 17 patients (36.2 %). Abnormalities consisted of abnormality including +8 (3 cases), complex abnormality with deletion 5 (2 cases), and complex abnormality with 20q− (3 cases). Chromosomal abnormalities in RCMD were found in bone marrow samples from 27 RCMD patients (39.7 %). Abnormality including +8 was detected in nine cases (33.3 %) and abnormality of idic (X) (q13), associated with the *ABCB7* gene [17], was found in one case. In addition, −7, which was not identified in RARS, was identified in four RCMD patients (14.8 %).

Treatment and outcome

Analysis of the available data regarding treatment indicated that 17 of 47 RARS cases and 26 of 72 RCMD cases were administered Vit.B6 (data not shown). The effectiveness was judged according to the criteria of IWG [18], and one RARS patient obtained a major response, and three RARS patients and one RCMD patient obtained minor responses. Thus, 4 of 17 RARS patients and 1 of 26 RCMD patients responded to Vit.B6 treatment. However, improvement of Hb was not sustained in two RARS patients; Hb level gradually returned to or dropped below the pretreatment level. Therefore, Vit.B6 treatment may not be effective for MDS, or the effect if any may be very limited. The clinical outcomes of patients are shown in Supplemental Table 1. The median follow-up from the time of diagnosis in CSA patients was 30.5 months, and two patients died due to sepsis (one case) and cardiac failure (one case). One patient

Table 2 Clinical data of CSA, RARS, and RCMD (2)

	CSA (n=18)	RARS (n=47)	RCMD (n=72)	p-value (between CSA and RARS)	p-value (between CSA and RCMD)
Total bilirubin (mg/dl)	1.1 (±0.8)	1.3 (±0.9)	1.1 (±0.7)	0.47	0.78
AST (GOT) (IU/l)	33.0 (±24.3)	24.9 (±11.7)	27.9 (±20.8)	0.08	0.38
LDH (IU/l)	218.3 (±98.9)	263.5 (±119.2)	246.1 (±97.7)	0.16	0.28
CRP (mg/dl)	0.13 (±0.15)	0.40 (±1.16)	1.17 (±3.81)	0.37	0.30
Serum iron (mg/dl)	210.7 (±77.6)	162.8 (±73.6)	171.1 (±66.2)	0.03	0.04
UIBC (mg/dl)	80.4 (±113.6)	102.4 (±82.7)	79.9 (±60.7)	0.48	0.93
Ferritin (ng/ml)	1239.8 (±1306.8)	743.4 (±815.3)	804.3 (±990.2)	0.08	0.13

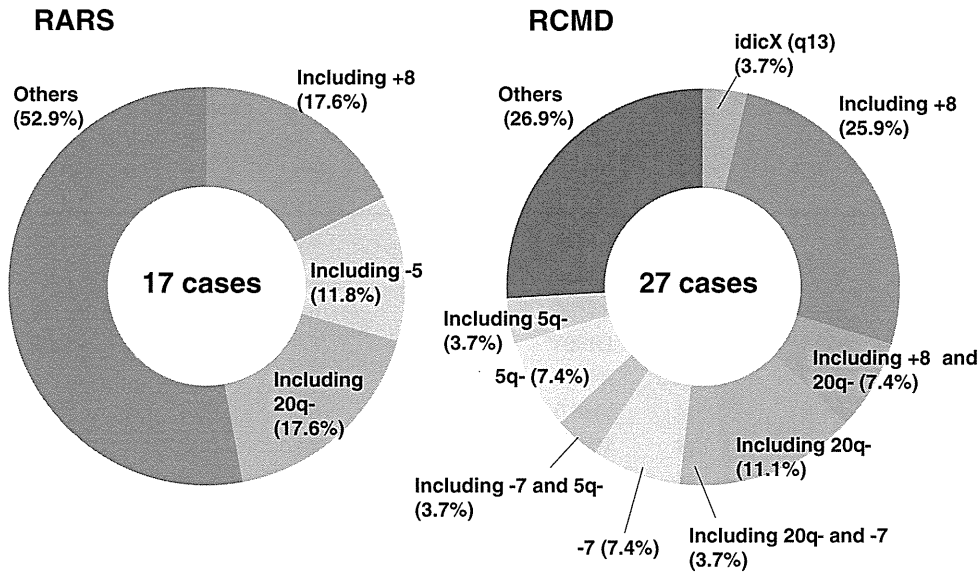

Fig. 1 Chromosomal abnormalities in RARS and RCMD. Data of chromosomal analysis in RARS and RCMD are shown. +8 was most common both in RARS and RCMD. -7 was only seen in RCMD

who died due to cardiac failure was heavily iron overloaded as defined by serum ferritin level, suggesting that cardiac complications may be caused by hemochromatosis. The median follow-up from the time of diagnosis in RARS patients was 23 months, and 6 patients (12.8 %) died due to pneumonia (two cases), evolution to leukemia (one case), and others (three cases). The median follow-up from the time of diagnosis in RCMD patients was 19.5 months, and 20 patients (27.8 %) died due to pneumonia (7 cases), cardiac failure (3 cases), evolution to leukemia (2 cases), sepsis (1 case), and others (7 cases). These results suggest that the prognosis of RCMD is worse than that of RARS.

Gene analysis of congenital sideroblastic anemia

Eighteen CSA patients were candidates for gene analysis; however, mutation analysis for genes responsible for CSA was not performed in four patients. One patient was diagnosed as having PMPS based on clinical findings, and DNA samples were not available for the remaining three patients. Therefore, gene analysis was performed in 14 of 18 CSA patients. Ten of these 14 patients were diagnosed as XLSA due to *ALAS2* mutation. Table 3 summarizes the results of gene analysis in XLSA. Case 2 (R411C), case 4 (D190V), case 6 (M567I), and case 7 (V562A) were reported previously [19–21]. Since amino acid substitution at Arg170, 411, and 452 were observed in plural patients, there are hot spots of mutation of *ALAS2* gene.

Patient with D190V (case 4), R170L (Case 10) and two patients with R452C (cases 3 and 5) did not respond to Vit.B6 treatment, whereas six patients responded to Vit.B6 treatment, although the increment of hemoglobin varied from 1.7 to 8.1 g. Interestingly, case 8 responded to Vit.B6 treatment, whereas case 10 did not, although both of them harbor the same mutation, R170L. Therefore, the activity of R170L mutant proteins was examined to determine the property, especially the Vit.B6 responsiveness. The enzymatic activities of wild type and R170L mutant protein were 7,193±138 nmol ALA/mg protein/h and 2,240±145 nmol ALA/mg protein/h, respectively, in the absence of PLP (Fig. 2). With an excess amount of PLP (100 μM) in the assay mixture, higher enzymatic activities were obtained with wild-type and mutant proteins (12,662±311 nmol ALA/mg protein/h and 7,700± 49 nmol ALA/mg protein/h, respectively) (Fig. 2). In addition, the enzymatic activity of R170C, which is another substitution at Arg170 found in this study, was also examined. As shown in Fig. 2, The enzymatic activity of mutant protein was significantly lower than wild-type protein without PLP (4,612± 87 nmol ALA/mg protein/h vs 7,193±138 nmol ALA/mg protein/h), and the activity was restored by addition of excess amount of PLP (100 μM) in the assay mixture. These in vitro data suggest that amino acid substitution at Arg 170, at least R170L and R170C, results in the decrease in enzymatic activity, but the decrease can be recovered by excess amount of PLP. The enzymatic activity of mutant proteins, which were identified in this study, is summarized in Table 3. The enzymatic activities of R411C, D190V, M567I, and V562A were referred from previous reports [19–21]. The levels of activity and PLP responsiveness in vitro were not correlated with clinical responsiveness to PLP in some cases. It is possible that the variety of mechanisms, such as the decrease in enzymatic activity of mutant ALAS2 protein, the changes of amount of *ALAS2* transcript, and physiological and environmental status of the patients, are responsible for the development of the disease.

Data for CSA patients other than XLSA are summarized in Table 4. Case 15 was diagnosed as PMPS. Gene analysis was not performed for cases 16 and 17; however, XLSA was strongly suspected because these patients were male and had microcytic anemia that was responsive to Vit.B6 treatment.

Table 3 Congenital sideroblastic anemia (XLSA)

Case number	Age at diagnosis (y.o.)	Gender	Position of *ALAS2* mutation	*SF3B1* mutation	Hb at onset (g/dl)	MCV at onset (fl)	Increment of Hb by Vit.B6 treatment (g/dl)	In vitro enzymatic activity of mutant protein[a]	
								Without PLP	With PLP
1	0	M	R170C	N/D	4.8	52.5	1.7	64.1 %	72.5 %[b]
2	20	M	R411C	N/D	4.8	52.5	5.2	11.9 %	25.1 % [19]
3	68	M	R452C	–	6.0	67.3	No effect	99.9 %	94.0 % [21]
4	17	M	D190V	N/D	8.9	66.9	No effect	98.6 %	98.5 % [20]
5	36	M	R452C	–	7.4	70.0	No effect	99.9 %	94.0 % [21]
6	36	M	M567I	N/D	6.5	64.4	3.4	38.1 %	25.2 % [21]
7	14	M	V562A	–	8.1	61.2	4.7	150.6 %	116.9 % [21]
8	31	M	R170L	–	4.1	50.8	8.1	31.1 %	60.8 %[b]
9	3	M	R411C	–	5.4	54.4	2.9	11.9 %	25.1 % [19]
10	62	M	R170L	N/D	8.0	73.9	No effect	31.1 %	60.8 %[b]

[a] % of WT

[b] Present study

ALAS2 mutations were not identified in cases 11, 12, 13, and 14. Therefore, mutations of *SLC25A38*, *GLRX5*, *ABCB7*, *PUS1*, *SLC19A2*, and *SF3B1* were examined in these cases; however, no mutations were identified in these cases. In contrast to other cases, case 18 was female and showed normocytic anemia. She was diagnosed with CSA due to family history; however, gene mutation analysis was not performed because DNA samples were not available. *SF3B1* gene mutation was examined in nine cases including five XLSA, however, no mutation was identified (Tables 3 and 4). On the other hand, *SF3B1* gene mutation was frequently detected in MDS-RS (Table 5).

Discussion

Because of its rarity, there have been few clinical and pathological investigations focusing on sideroblastic anemia. This study was performed to investigate the epidemiological and pathological characteristics of sideroblastic anemia. Based on the data of 137 patients, it was revealed that hemoglobin level in CSA was significantly lower than those seen in MDS, and serum iron level was higher in CSA compared to MDS. These results revealed that anemia in CSA is more severe than that in MDS at onset, although significant cases improved by Vit.B6 treatment. Reflecting the high incidence of XLSA in CSA, MCV level was significantly lower in CSA than MDS. These findings suggest that CSA should be strongly suspected rather than MDS, at least in Japan, in male patients exhibiting microcytic anemia and an elevated serum iron level.

MDS-RCMD is the most common form of acquired sideroblastic anemia. Chromosomal abnormalities were observed in 39.7 % of RCMD cases and 36.2 % of RARS cases. The types of chromosomal abnormality frequently observed in RCMD and RARS did not differ from those reported previously, such as +8, −7, 20q− and −5. Among them, +8 was observed in nine cases of RCMD (33.3 %). As the frequency of +8 in MDS was reported to be 6.5–16.7 %,

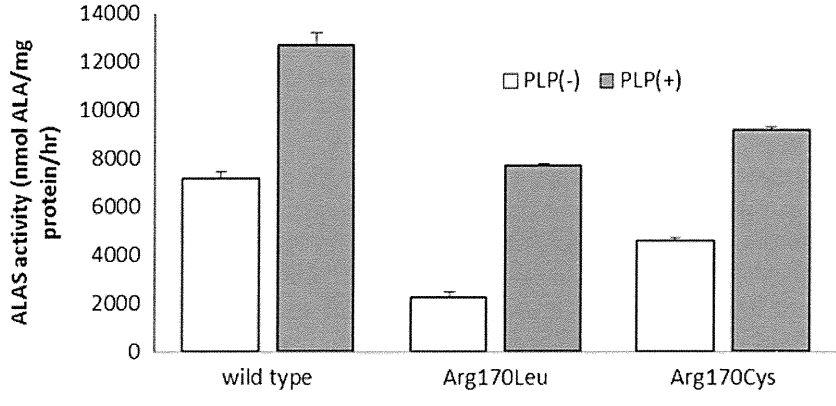

Fig. 2 Enzymatic activity of mutant ALAS2 proteins. Enzymatic activity of wild-type and mutant ALAS2 proteins was measured as described in Materials and Methods. Both of R170L and R170C ALAS2 mutant proteins showed decreased enzymatic activity; however, the activity was partially restored by the addition of PLP

Table 4 Congenital sideroblastic anemia (other than XLSA)

Case number	Age at diag (y.o.)	Gender	Family history	Gene mutation							Hb (g/dl)	MCV (fl)	Response to Vit.B6
				ALAS2	*SLC25A38*	*GLRX5*	*ABCB7*	*SLC19A2*	*PUS1*	*SF3B1*			
11	19	M	−	−	−	−	−	−	−	−	7.8	73.9	−
12	4	M	−	−	−	−	−	−	−	−	6.6	73.6	−
13	0	M	+	−	−	−	−	−	−	−	3.9	65.0	−
14	20	M	+	−	−	−	−	−	−	−	7.6	82.0	+
15	0	M	−	N/D	N/D	N/D	N/D	N/D	N/D	N/D	6.8	88.1	N/D[a]
16	32	M	−	N/D	N/D	N/D	N/D	N/D	N/D	N/D	11.2	69	+
17	36	M	−	N/D	N/D	N/D	N/D	N/D	N/D	N/D	10.8	67.3	+
18	18	F	+	N/D	N/D	N/D	N/D	N/D	N/D	N/D	9.3	96.2	+

N/D not done

[a] Vit.B6 was not administered due to PMPS

+8 appeared to be more common in RCMD. In addition, −7 was identified in four patients with RCMD (14.8 %), whereas it was not identified in RARS. This difference may be related to the poor prognosis of RCMD.

Regarding the responsiveness to pyridoxine treatment among XLSA, 6 of 10 cases responded to Vit.B6 treatment in this study, although the magnitude of response varied among individuals. Thus, as the benefit of treatment of Vit.B6 for XLSA is obvious, a precise diagnosis of XLSA is important. As late-onset XLSA cases have been reported and two patients over 60 years old were found in this study, genetic analysis in sideroblastic anemia patients with microcytic anemia is essential regardless of age.

Focusing on *ALAS2* mutation in XLSA, two patients with the same mutation (c.509G>T), which results in R170L, showed distinct responses to Vit.B6. Edgar et al. [22] reported a Vit.B6 responsive pedigree with XLSA carrying the p.R170L mutation of ALAS2 gene. Furthermore, the crystal structure analysis of ALAS from *Rhodobacter capsulatus* [23] suggests that a missense mutation at Arg170 destabilizes PLP binding, which might be partially restored with excess amounts of PLP. Together with the findings of biochemical analysis in this study, it is strongly suggested that R170L mutation causes pyridoxine-responsive XLSA. However, in consistent with the data of in vitro analysis and clinical course of other R170L patients, case 10 was unresponsive to Vit.B6 treatment. Thus, onset and severity of the disease may be defined by not only the type of mutation but also the environmental and physiological status of the patients. This speculation may be supported by the results that there is a discrepancy between in vitro and in vivo response to Vit.B6 in some cases (Table 3).

The high incidence of XLSA among CSA in the present study was consistent with a previous report in the USA. Bergmann et al. [24] reported genetic analysis of CSA in the USA. In this study, mutations of *ALAS2*, *SLC25A38*, mitochondria DNA, and *PUS1*, were identified in 37, 15, 2.5, and 2.5 % of CSA cases, respectively. The most significant difference from our study was that mutations of the *SLC25A38* gene were frequently found in the USA. Since *SLC25A38* is thought to be a transporter of glycine, which is a substrate for ALAS2 in the first step of heme synthesis, the

Table 5 Mutation of *SF3B1* gene in MDS-RS

Case number	Diagnosis	Age at diagnosis (y.o.)	Gender	Chromosome anomaly	position of *SF3B1* mutation
1	RARS	82	M	−	E622D
2	RARS	57	M	−	N626S
3	RARS	60	M	Complex karyotype, including +8	K700E
4	RARS	60	M	−	K700E
5	RARS	73	F	−	No mutation
6	RARS	74	F	−	H662Q
7	RARS	76	M	−	K700E
8	RARS	67	F	−	K700E
9	RARS	66	M	−	K666E
10	RCMD	50	F	−	No mutation

(−) normal karyotype

pathology of CSA due to mutation of this gene is similar to that of XLSA. Therefore, CSA patients with microcytic anemia, in whom mutations of *ALAS2* gene were not identified, were expected to harbor *SLC25A38* mutation; however, it was not detectable in this study. To date, it has not been reported in Asia, although mutation of the *SLC25A38* gene has been widely reported in the USA, Canada, and Europe. Together with the results of the present study, it is suggested that the causative genes of CSA differ among races and regions.

Recently, mutations of genes involved in splicing machinery were reported in MDS [6]. Among them, *SF3B1*, which is a component of the U2-small nuclear ribonucleoprotein (U2-snRNP) complex [25], was found to be highly mutated in MDS with ring sideroblasts [6]. In this study, *SF3B1* mutation was examined in nine cases of CSA; however, its mutation was not detectable in CSA. These findings suggest that the mechanism for sideroblasts formation may be different between CSA and MDS.

In conclusion, our data showed that XLSA is the most frequent type of CSA; however, onset and severity of the disease may be affected by the environmental and physiological status of the patients. The data, including clinical and genetic analysis, further suggest that genetic background is different between CSA and MDS.

Acknowledgements We thank the doctors participating in this study for providing clinical data and samples. This research was supported by a Grant-in-Aid for Scientific Research from the Ministry of Health, Labor, and Welfare of Japan.

Conflict of interest disclosure The authors declare no competing financial interest.

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日本の遺伝性ポルフィリン症
～1920年（第1例報告）から91年間（2010年）の集計～

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1920年に国内で初めて遺伝性ポルフィリン症が報告されてから2010年12月末までの91年間に926症例のポルフィリン症患者報告例を見出した。そこで、この926症例について、病型別に年齢・性・地理的分布、発症要因、臨床症状、初期診断、治療および予後などの集計を行った結果、本邦の遺伝性ポルフィリン症の年度別発症動向や臨床症状、誘発要因などの実態が解明され、難病としてのポルフィリン症の全体が展望され、ポルフィリン症研究において大変貴重なデータを得ることができた。しかし、本研究によって見出された症例は発症者の報告であり、遺伝的素質を持ちながら未発症者または医師が報告していない症例、および誤診症例などは含まれていない。

本研究では確定診断された926例について、原著論文や症例報告に記載してある内容について詳細にまとめた。本症は世界中に存在し、その希少性および深刻な症状から注目されてきたが、国外でのポルフィリン症に関する統計報告は殆ど見当たらず、その実態については殆ど不明であった。以上のことから、今回の調査結果は国際的にも非常に貴重な報告であり、ポルフィリン症の実態解明が急速に進むことが期待される。

Key Words：ポルフィリン症、肝性ポルフィリン症、赤芽球性ポルフィリン症、皮膚型ポルフィリン症、急性ポルフィリン症

はじめに

ポルフィリン症を「病気の主座がポルフィリン代謝の異常にある一群の疾患」と定義する。本症は他の先天性代謝異常症と同じく極めてまれな疾患であるが、その特異的な症状のため、古くから（世界最初の報告例は1876年[1]）知られ、注目されてきた。本症は1908年にAE Garrod[2]により先天性代謝異常症の代表的疾患として取り上げられて以来、現在までに8病型が知られている[3-5]。また、最近δ-アミノレブリン酸合成酵素(ALAS2)のC末端側にdeletionを持ち、光線過敏症を有する8家系のポルフィリン症が見いだされ、X連鎖優性プロトポルフィリン症(XLDP)[6]と命名されたが、XLDPについての情報は少ない。

ポルフィリン症の分類は酵素異常がどの臓器に発現するかによって、肝性と赤芽球（または骨髄）性に大別される（表1）。しかし、一般的には皮膚の光線過敏症状を主とする皮膚型ポルフィリン症（先天性赤芽球性ポルフィリン症；CEP、赤芽球性プロトポルフィリン症；EPP、肝赤芽球性ポルフィリン症；HEP、晩発性皮膚ポルフィリン症；PCT）と急性の神経症状を主とする急性ポルフィリン症（急性間欠性ポルフィリン症；AIP、多様性ポルフィリン症；VP、遺伝性コプロポルフィリン症；HCP、δ-アミノレブリン酸脱水酵素欠損性ポルフィリン症；ADP）として分類されることが多い[3-5]。

本研究では、大正9年(1920)に報告された第1例[7]から2010年12月までに、ポルフィリン症として報告された926症例について、本邦における

表1 ポルフィリン症の分類と特徴

ポルフィリン症				酵素	遺伝	主要症状		生化学的所見
分類		病型*	略称	異常	形式	皮膚症状	神経症状	ポルフィリン代謝産物の異常増量
赤芽球性肝性	皮膚型	先天性赤芽球性ポルフィリン症	CEP	UROS	劣性	+++	−	尿、血液中UP I
		赤芽球性プロトポルフィリン症	EPP	FECH	優性	+〜++	−	血液中FP
		肝赤芽球性ポルフィリン症	HEP	UROD	劣性	+++	−	尿中UPIII、血液中ZP
		晩発性皮膚ポルフィリン症(家族性)	fPCT	UROD	優性	+〜+++	−	尿中UPIII、糞中isoCP
		晩発性皮膚ポルフィリン症(散発性)	sPCT	UROD	不明	+〜+++	−	尿中UPIII、糞中isoCP
	急性	多様性ポルフィリン症	VP	PROX	優性	+〜++	++	尿中ALA, PBG, UPIII、糞中PP、XP
		遺伝性コプロポルフィリン症	HCP	CPO	優性	−〜++	++	尿中ALA, PBG, CPIII、糞中CP
		急性間歇性ポルフィリン症	AIP	PBGD	優性	−	++	尿中ALA, PBG
		ALAD欠損性ポルフィリン症	ADP	ALAD	劣性	−	++	尿中ALA

＊分類名称はポルフィリン研究会にて統一した名称を用いた。FP：赤血球遊離プロトポルフィリン、ZP：亜鉛結合型プロトポルフィリン

ポルフィリン症の実態を把握すべく、病型別に諸情報を整理し、年齢・性・地理的分布、発症要因、臨床症状、初期診断、治療および予後などにつき新知見を投入しながら統計学的検討を行った。

なお、本報告に関しては、これまでに1920年から各々1982年[8]、1992年[9]、1998年[10]、2000年[11]、2002[12]、2008年[13]と、常に新しい情報を取り入れ報告してきたが、著者らによる本調査研究は本論文にて終了したい。

I．研究方法

2010年12月までに医学中央雑誌に遺伝性ポルフィリン症として記載されたすべての報告について、抄録や原著論文を一つひとつ精査し、その中から、ポルフィリン症として記載するのが適当でないと思われたもの（例えば、他疾患に併発した一過性のポルフィリン尿症[14]、ポルフィリン症の診断基準をみたしていないもの、ポルフィリン症の可能性が高いが検査所見[15,16]の記述が不十分なものなど）を除き、これに著者らによる未発表の自験症例を加え、日本国内で報告された日本人の全報告例についてまとめた。ADPについては1979年Dossら[17]によって初めて報告されて以来、世界で6例の報告しかなく、極めてまれな疾患である。国内では1979年に1例のADP症例が報告されたが[12]、ポルフィリン代謝関連測定結果情報不足のため除いた。

II．結果および考察

1．疫学統計〜患者の動向〜

1）病型別・性別・報告年代別頻度

遺伝性ポルフィリン症として確定した926例につき、病型別、性別および報告年代ごとにまとめた（表2）。年代区分は、ポルフィリン生合成系が解明されるまでを〜1955年、ヘム生合成系の解明からALAS測定による酵素学的診断の幕開けまで

表2 日本におけるポルフィリン症患者報告例数（病型別，年代別）

1920年1月〜2010年12月

病型別	1920〜1955	1956〜1965	1966〜1975	1976〜1985	1986〜1995	1996〜2005	2006〜2010	計	本邦第一例報告年
CEP	12(3: 9)	4(2: 2)	10(5: 5)	3(2: 1)	4(2: 2)	1(1: 0)	2(0: 2)	36(15:21)	1920[7]
EPP	0(0: 0)	2(2: 0)	23(13:10)	39(26:13)	42(28:14)	64(39:25)	33(29: 4)	203(137:66)	1964[18]
小計	12(3: 9)	6(4: 2)	33(18:15)	42(28:14)	46(30:16)	65(40:25)	35(29: 6)	239(152:87)	
AIP	2(0: 2)	36(9:27)	65(11:54)	31(3:28)	31(4:27)	28(5:23)	5(0: 5)	198(32:166)	1932[19]
VP	0(0: 0)	9(1: 8)	17(5:12)	11(0:11)	9(1: 8)	8(3: 5)	2(0 :2)	56(10: 46)	1962[20]
HCP	0(0: 0)	0(0:0)	21(3:17)*1	1(1: 0)	4(3: 1)	11(3: 8)	4(3: 1)	41(13: 27)*1	1966[21]
分類不明†	8(2: 6)	11(3: 8)	7(2: 5)	10(3: 7)	13(3: 10)	6(3: 2)*1	3(1: 2)	58(17: 40)*1	
小計	10(2: 8)	56(13:43)	110(21:88)*1	53(7:46)	57(11:46)	53(14:38)*1	14(4: 10)	353(72:279)*2	
PCT	0(0: 0)	3(3: 0)	41(39: 2)	145(137: 5)*3	76(65:11)	43(40: 3)	20(16: 4)	328(300: 25)*3	1957[22]
HEP	0(0: 0)	0(0: 0)	1(1: 0)	3(2: 1)	0(0: 0)	1(1: 0)	1(0: 1)	6(4: 2)	1972[23]
小計	0(0: 0)	3(3: 0)	42(40: 2)	148(139: 6)*3	76(65:11)	44(41: 3)	21(16: 5)	334(304: 27)*3	
計	22(5:17)	65(20:45)	185(79:105)*1	243(174:66)*3	179(106:73)	162(95:66)*1	70(49:21)	926(528:393)*5	

†原論文から得られた情報が不足のため，AIP，VP，HCPのいずれかに決定ができなかったものをこの項に集めた．
したがって，この型は本稿だけ用いられる便宜的なものである．＊性別不明(数字は例数を示す)、(男：女)
CEP：先天性赤芽球性ポルフィリン症、EPP：赤芽球性プロトポルフィリン症、AIP：急性間歇性ポルフィリン症、VP：異型ポルフィリン症
HCP：遺伝性コプロポルフィリン症、PCT：晩発性皮膚ポルフィリン症、HEP：肝赤芽球性ポルフィリン症

の時期を 1956～1965 年、生合成系各段階の酵素学的研究の進歩により、ほぼ現在の病型分類が確定した時期を1966～1975年とした。その後の1976～1985 年および 1986～1995 年は高速液体クロマトグラフィーによるポルフィリン症の生化学的診断および酵素活性測定による病型診断が比較的ポピュラーになり、診断がより厳密になると共に、ポルフィリン代謝系の病態解明が盛んとなった時代といえ、1996 年以降は他の遺伝性疾患と同様に遺伝子診断による時期といえる。

報告年代と病型別頻度の推移を見ると（図 1)、各病型とも本邦での第１例報告年次から報告数が増加しているが、特に、ポルフィリン生合成系の解明と同時に各病型のポルフィリン症の報告数が増加し、1966～1975 年では急性ポルフィリン症の報告が主流となり、それにやや遅れて EPP、PCT などの皮膚型ポルフィリン症の報告が増加している。

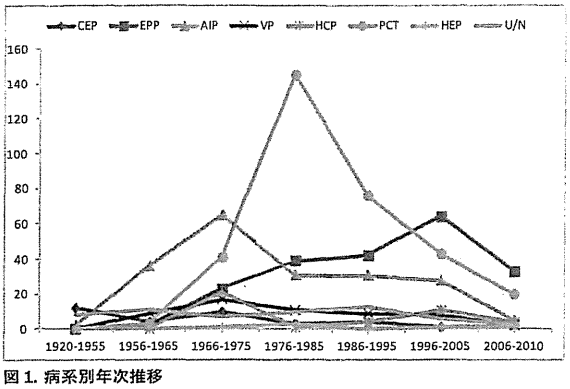


図 1. 病系別年次推移

しかし、1976 年以降、急性ポルフィリン症各型の報告数が少なくなっている。最近の５年間では 14 症例の報告があるのみである（表 2)。これは本症の発症数の減少を示すものではなく、すでに単なる症例報告のみでは報告する価値が認められない時代に入っていることを反映していると考えられる。または、希少疾患ということで誤診されているものと思われる。したがって、未報告例、潜在例を含めた実数は、本論文に現れたものの数倍に達するものと思われる。しかし、希少疾患であることは疑いえない。

なお、926 例中 58 例の急性ポルフィリン症についてはデータ不足により分類不明型の急性ポルフィリン症とした（表 2)。

患者の各病型別頻度（図２）では PCT が 35%、EPP が 22%であり、全報告の 50%以上が皮膚型ポルフィリン症であった。急性ポルフィリン症の中では AIP が 21%、VP、HCP が 4～6%であった。HEP に関しては ADP と同様極めてまれな疾患で、肝性と赤芽球性の双方の生化学的性質を持つことから、EPP の肝障害タイプとよく間違われる事がある。表２より、2010 年までに報告された HEP の６例については UROD 酵素（活性または遺伝子）異常について、またはポルフィリン代謝関連物質についての十分な検討が成されていない。1999 年に我々は HEP が強く疑われた症例のポルフィリン代謝異常を報告した[24]。

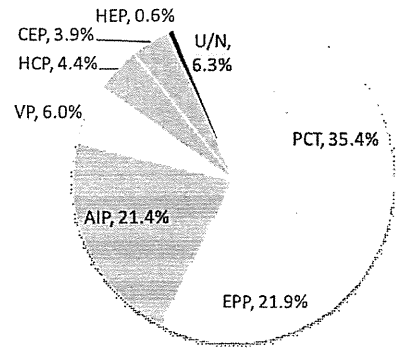
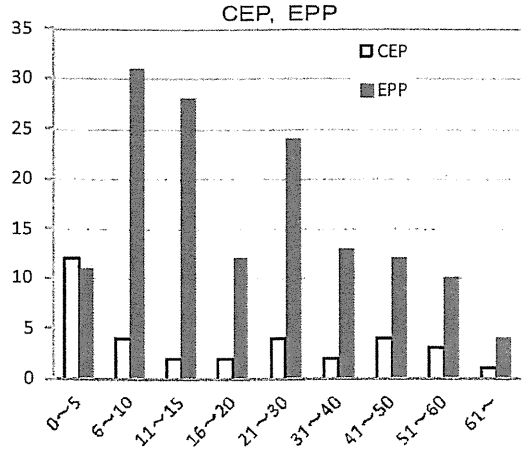


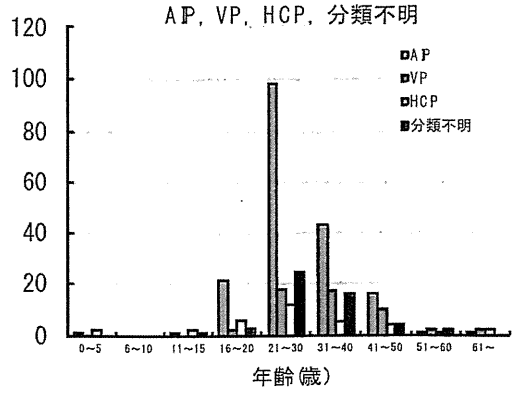
図 2. 各病系別患者頻度

２）年齢別報告数

本症は遺伝性疾患でありながら病型ごとに年齢別発症頻度が異なっていた（図 3)。すなわち、CEP の約半数が幼年～若年に多く、この内７例は 16～40 歳に発症しており、注目に値する（我々は CEP 全症例の詳細な報告を行った[25])。EPP は 6～30 歳に多く（男＞女)、また、PCT は 30 歳以降の男性に多かった。急性ポルフィリン症では思春期から妊娠可能な中年期の女性に多く見られた。CEP を除いていずれも常染色体優性に遺伝することが知られているが、このような年齢差、性差は本症発症機構および発症の予防を考える上で重要であり、急性ポルフィリン症に代表されるごとく、本症の発症・増悪には遺伝的障害のほかに、

別の多くの誘発因子が加わることが重要である。





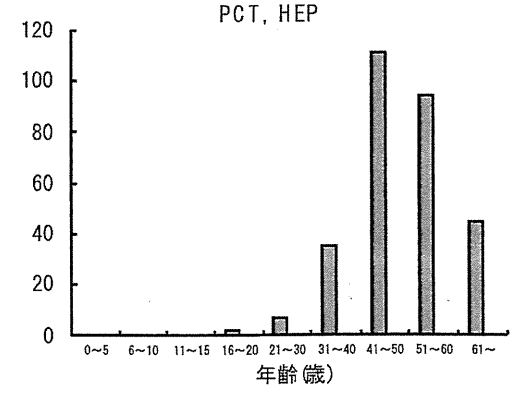


図3 ポルフィリン症の年齢別頻度

3）同胞発症・血族結婚の頻度

本症は遺伝的疾患にもかかわらず、血族結婚も同胞発症も見られない弧発例の報告が多い（表 3）。これは、個々の報告の不完全さ、論文による資料面での検討という制約にもよるが、著者らの経験からしても、潜在患者（不顕性遺伝子保有者）のまま無症候性に経過する同胞が少なくないことも事実であり、ここでも誘発因子の有無が重要な意味を持っていると考えられる。PCT に関しては、わが国では同胞発症の報告は少なく、これまでに同胞発症を見たものは3例にすぎない。しかし、本邦での PCT の全症例について遺伝子の検討は行われておらず、今後、遺伝子機構、発症要因も含めて本症の発症機序について詳細な検討が必要である[26]。

表3 同胞発症と血族結婚の頻度

	同胞発症 +		同胞発症 −	
	血族結婚 +	血族結婚 −	血族結婚 +	血族結婚 −
CEP	2	16	4	12
EPP	1	78	0	75
AP	8	73	4	107
VP	0	8	1	45
HCP	1	16	1	19
AP	0	6	0	45
PCT	0	3	1	299
HEP	0	3	0	3

4）ポルフィリン症の地理的分布

都道府県別分布（表 4）は、各病型とも全国から報告されているが研究者、医療施設の分布による偏りがかなりあると考えられる。すなわち、東京、神奈川、新潟、大阪、長崎など、ポルフィリン症研究者のいる都府県で多くの報告が見られる。多くの論文では患者の出身地ではなく、症例報告者の所属する都道府県になっているので注意が必要であり、患者の地理的分布の実態は不明である。

表4 ポルフィリン症の地理的分布

	病型 赤芽球型	急性型	皮膚晩発型	計
北海道	1	7	20	28
青森県	3	1	6	10
岩手県	0	0	3	3
宮城県	4	9	14	27
秋田県	2	6	5	13
山形県	0	4	0	4
福島県	2	2	3	7
茨城県	1	1	1	3
栃木県	3	2	3	8
群馬県	2	7	3	12
埼玉県	2	3	1	6
千葉県	7	5	2	14
東京都	42	63	39	144
神奈川県	10	18	23	51
新潟県	2	40	5	47
富山県	1	2	1	4
石川県	11	4	6	21
福井県	0	1	1	2
山梨県	0	0	3	3
長野県	2	8	8	18
岐阜県	0	6	1	7
静岡県	4	4	3	11
愛知県	9	12	5	26
三重県	8	6	14	28
小計	116	211	170	497

	病型 赤芽球型	急性型	皮膚晩発型	計
滋賀県	0	0	1	1
京都府	7	9	8	24
大阪府	9	25	14	48
兵庫県	7	6	13	26
奈良県	0	0	3	3
和歌山県	4	2	2	8
鳥取県	1	13	7	21
島根県	0	2	1	3
岡山県	4	10	6	20
広島県	6	2	10	18
山口県	0	3	12	15
徳島県	2	4	1	7
香川県	2	5	2	9
愛媛県	3	0	1	4
高知県	2	5	5	12
福岡県	19	14	18	51
佐賀県	1	0	3	4
長崎県	21	8	40	69
熊本県	8	6	2	16
大分県	0	6	1	7
宮崎県	3	6	5	14
鹿児島県	21	12	5	38
沖縄県	3	3	3	9
県不明	0	1	1	2
小計	123	142	164	429
総計	239	353	334	926

赤芽球性：CEP, EPP、 急性：AP, VP, HCP, 分類不明、 皮膚晩発性：PCT, HEP

一方、国際的に、英国の患者報告数と比較した結果（表 5）[27]では、各病型において患者の発現頻度が一致していたことから、地

表5 ポルフィリン症患者数の日英比較

	日本(例数)	英国(例数)	日本 %	英国 %
PCT	303	949	37	39
AIP	188	599	23	25
EPP	154	356	19	15
VP	54	193	7	8
HCP	37	40	4	2
CEP	34	36	4	2
Others	57	255	7	9
Total	827	2428	100	100

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ポルフィリン症は世界中に存在するが、発見されているのは1例にも満たない。表は1992年時点での比較である。

理的偏りは殆どないものと思われる。したがって、この地理的分布ではかなりの頻度にて、診断し得ていない患者が多数存在するものと思われる。

2．臨床統計～臨床症状、誘因、診断、治療及び予後～

ポルフィリン症の臨床症状の特徴から、皮膚型ポルフィリン症は皮膚科で見いだされることが多いが、内科（消化器内科）、小児科（血液、消化器内科）で発見されることも少なくない。一方、急性ポルフィリン症は神経内科、消化器科、精神科などを初診とすることが多く、一部は急性腹症として救急外来、外科、婦人科を最初に受診することも少なくない。

1）皮膚型ポルフィリン症の臨床症状

皮膚型ポルフィリン症は蓄積するポルフィリン体の種類・量によって皮膚症状の程度が大きく異なり、CEP が最も激しく、次いで PCT 、EPP の順である。CEP では皮膚病変以外に多毛・剛毛、爪の変形、耳、鼻や指の部分的欠損、赤色歯牙、脾腫が注目される（表 6）。PCT では肝障害がほぼ

表6 皮膚型ポルフィリン症の臨床症状

	CEP (36例中)	EPP (203例中)	PCT (328例中)	HEP (6例中)	計 (573例中)
皮膚症状					
日光過敏症 紅斑	33	135	71	2	241
水疱, びらん	31	35	208	0	274
潰瘍	27	24	83	0	134
痂皮	16	19	114	1	150
瘢痕	34	64	186	1	285
色素沈着	34	64	206	1	305
脱失	8	1	43	0	52
肥厚・強皮症様瘢痕	16	14	18	0	48
脆弱性	13	6	92	0	111
多毛・剛毛	16	2	42	0	60
脱毛	6	1	3	0	10
骨軟骨の欠損脱落					
(爪の変形, 鼻・耳・指の欠損)	19	4	5	0	28
赤色歯牙	23	1	0	0	24
赤色尿	32	1	71	0	104
貧血	9	11	7	2	29
脾腫	8	5	6	1	20
肝 肝硬変	0	8	15	0	23
肝機能障害	7	47	299	6	359
糖代謝異常	0	0	20	0	20
その他(消化器, 神経症状など)	0	9	10	2	21

必発であり、大多数の症例で健常者との有意差は認められないが、血清鉄の上昇を認める[28]。また、表 7 に示したように、PCT 患者の約 29%(98 例/337 例）の肝生検による組織学的所見では慢性肝炎、肝硬変、肝癌が多い。さらに、1987 年に C 型肝炎ウィルス(HCV)の抗体検査が可能になって以来、PCT 患者のC型肝炎合併の報告例が高率（我々の調査では 85%に陽性が見られた[29,30]）で見られ、詳細は不明であるが、その因果関係は今後の問題点の一つである。

表7 PCT(337例)中、肝生検を施行した98例の病理組織学的診断

	98例中	HCV抗体陽性例 (61例中)
肝硬変	15	
慢性肝炎		
活動型	24	14
非活動型	12	2
アルコール型	12	5
その他	26	
肝 癌	13	1
脂肪変性	20	1
詳細不明	7	24
合 計	129	47

129例については重複診断あり

2）急性ポルフィリン症の臨床症状

急性ポルフィリン症は多彩な症状が種々の組合せで、急性または亜急性に出現し、増悪・寛解を見るのが特徴である。表 8 にその自覚的初発症状を、表 9 に初診時の他覚的所見を、表 10 に全経過中に見られた症状をまとめた。

表8 急性ポルフィリン症の自覚的初発症状

	AIP (198例中)	VP (56例中)	HCP (41例中)	分類不明 (58例中)	計 (353例中)
消化器症状					
腹痛	165	37	31	45	278
嘔吐	76	29	26	26	157
便秘	38	14	10	19	81
下痢	8	1	4	0	13
神経症状					
脱力および運動麻痺	25	18	13	6	62
四肢知覚障害	23	11	10	1	45
言語障害	1	4	1	1	7
嚥下障害	1	3	1	0	5
痙攣	5	1	4	5	15
意識障害	1	0	1	0	2
皮膚症状					
日光紅斑	0	14	3	1	18
色素沈着	0	11	4	0	15
暗褐色尿	35	16	12	11	74

表9 急性ポルフィリン症の他覚的症状(1)
—初発時症状の主たるもの—

	AIP (198例中)	VP (56例中)	HCP (41例中)	分類不明 (58例中)	計 (353例中)
腹部症状					
圧 痛	38	6	7	4	55
イレウス症状	34	0	3	2	39
神経症状					
末梢性運動麻痺	35	15	10	14	74
四肢知覚障害(表在性)	20	10	11	9	50
異常知覚	7	2	0	0	9
反射 亢進	4	1	2	1	8
減弱消失	14	7	6	8	35
意識障害	16	5	7	4	32
痙攣, てんかん発作	13	1	8	5	27
脳神経麻痺	8	4	2	2	16
膀胱直腸障害	7	1	0	0	8
精神障害					
幻覚, 妄想, せん妄	14	1	2	2	19
ヒステリー	4	0	1	0	5
循環器障害					
高血圧	23	14	10	4	51
頻脈	16	13	10	4	43
皮膚症状					
日光紅斑	0	7	3	0	10
色素沈着	1	13	3	1	18
その他					
肝障害	16	9	6	4	35
暗褐色尿	46	14	11	7	78

自覚的初発症状では神経症状よりは腹痛、嘔吐などの腹部症状が先行することが多いが、まれには意識障害、痙攣などで初発することがあり、表

には入れていないが、不眠とか不安感、あるいは胸部絞扼感とか腰背痛などが初発症状と考えられるものも見られた。また、集計には現れてこなかったが、分裂症、うつ症など精神症状として扱われているものの中に急性ポルフィリン症が見逃されていたという報告もあり、注意を要する。

全経過を通じての症状も従来の報告と変わるところはないが、例えば、腹痛だけで終始するという症例もあり、診断上、注意を要する。また、ポルフィリン症といえば、EPP 以外の病型ではすべて尿の着色が有名であるが、暗褐色（赤色）尿を見ることは比較的少なく 353 例中 132 例(37%)にすぎず（表 10)、注意しないと診断を誤ることになる。自律神経症状としては著明な発汗を認めるものが多く見られた。その他、比較的まれな症状として、デファンス、運動失調、低血圧、分裂病様精神症状などが見られた。

表10 急性ポルフィリン症の他覚的症状(2)
—全経過中にみられたもの—

	AIP (198例中)	VP (56例中)	HCP (41例中)	分類不明 (58例中)	計 (353例中)
腹部症状					
圧 痛	52	7	9	10	78
イレウス	42	2	4	4	52
神経症状					
末梢性運動麻痺	102	25	15	25	167
四肢知覚障害(表在性)	72	15	13	18	118
異常知覚	23	4	2	1	30
深部反射亢進	12	1	3	1	17
減弱消失	54	10	8	18	90
病的反射	5	0	2	2	9
意識障害	53	9	11	12	85
痙攣, てんかん発作	33	5	14	11	63
脳神経麻痺	28	5	5	5	43
球麻痺	40	8	1	13	62
膀胱直腸障害	39	2	1	1	43
筋萎縮	31	5	2	1	39
自律神経症状	30	8	4	5	47
精神障害					
幻覚, 妄想	51	4	3	4	62
ヒステリー	16	0	1	0	17
循環器障害					
高血圧	87	20	11	10	128
頻脈	85	17	10	7	119
内分泌代謝異常					
電解質異常(SIADH含む)	45	2	2	4	53
糖代謝異常	19	2	0	2	23
甲状腺機能異常	6	3	1	2	12
皮膚症状					
日光紅斑	0	10	3	0	13
色素沈着	1	19	3	1	24
爪・指などの変形・欠損	0	0	0	0	0
その他					
肝障害	45	13	6	7	71
暗褐色尿	90	19	11	12	132

3）発症、増悪の誘因

急性ポルフィリン症では種々の薬剤[31]、内分泌性因子、各種ストレス、その他により発症、増悪する（表 11)。とくに薬剤に関してはフェノバルビタール、ヒダントインなどの絶対的禁忌なものから、症例によって、安全とも禁忌とも報告されているものまで複雑であり、日常診療に際しては十分な注意が必要である。

PCT では 328 例中 253 例(77%）が飲酒歴を有し（表 11)、PCT の病因論上アルコールの占める役割が重要な課題となっている。諸外国では前立腺癌や更年期障害の治療、あるいは避妊目的でエストロゲン投与による発症例が多い[32]。また、後天性免疫不全症候群(AIDS)患者の PCT が多く[33]、HIV や HCV の感染は PCT の誘発因子となっている。EPP では強い紫外線曝露が誘因となる。

表11 発症, 増悪の誘因

	AIP (198例中)	VP (56例中)	HCP (41例中)	分類不明 (58例中)	PCT (328例中)	計 (681例中)
フェノバルビタール	24	7	12	3	1	47
解熱, 鎮痛, 鎮痙剤	26	1	1	2	3	33
妊娠, 月経, 分娩	33	2	2	2	1	40
ピ ル	6	1	1	0	0	8
眠 剤	3	1	0	0	0	4
アルコール	4	1	0	1	253	259
血液透析	0	0	0	3	9	12
その他	10	1	2	0	14	27
小 計	106	14	18	11	281	430
不 明	92	42	23	47	47	251

4）初期診断

ポルフィリン症は従来からまれな疾患とされ、また日常の臨床検査では発見されにくいことから、誤診や診断の遅れにより治療の時期を失することが少なくない[34]。とくに急性ポルフィリン症では皮膚症状の出るものは少なく、急激な腹痛にみまわれることが多いため、イレウス、虫垂炎、急性膵炎、結石などを含む急性腹症などと誤診されることが多く、これらの約 1/4 では誤って開腹手術を受けている。つわり、ヒステリー、Guillain-Barre 症候群なども十分に注意することが必要である（表 12)。

表12 初期診断(急性ポルフィリン症)

	AIP (198例中)	VP (56例中)	HCP (41例中)	分類不明 (58例中)	計 (353例中)
急性腹症	50	13	12	19	94
イレウス	28	3	3	5	39
虫垂炎	15	0	1	3	19
ヒステリー, 心因性反応	15	0	0	0	15
妊娠悪阻	6	1	1	2	10
急性膵炎	9	2	1	1	13
てんかん	2	0	7	3	12
急性胃炎, 胃・十二指腸潰瘍	4	0	2	2	8
肝障害	4	1	1	0	6
Guillain-Barre症候群	2	2	0	0	4
軸捻転(卵巣)	2	0	0	1	3
胆 石	1	1	0	0	2
子宮外妊娠	2	0	0	0	2
スモン	2	0	0	0	2
日光皮膚炎	0	1	1	0	2
腎・尿路結石	1	0	0	0	1
ミエロパチー	1	0	0	0	1
その他	1	0	2	0	3
総計	145	24	31	36	236

その他の中には脳炎、脳腫瘍、筋萎縮症、てんかん、潰瘍性大腸炎、心因性疼痛、うつ病、心身症、慢性収縮性心膜炎などの病名も見られた。皮膚型ポルフィリン症では致命的な誤診に至るものは少なく、CEP、EPP では初めに日光皮膚炎と診断されることが大部分であり、PCT では肝障害、肝硬変として診断されているものが少なくない。

5）治療と予後

（1）治療

皮膚型ポルフィリン症の治療内容を表 13 に示した。CEP では外用薬が、EPP では β- カロチンが、PCT では断酒、瀉血が遮光と共に治療の主体をなしている。PCT に関しては HCV との関与が示されるようになってからはインターフェロンの投与が試みられるようになってきている[35]。最近 10 年間で 16 例の PCT に投与され、肝機能の改善と同時に尿中ポルフィリンの減少が報告されているが、効果については必ずしも一定してなく、皮疹の抑制、鉄やポルフィリンの除去には瀉血療法[36]や鉄キレート剤であるデフェロキサミンの投与が有効[37]であるとする報告が多い。

表13 皮膚型ポルフィリン症の治療内容

	CEP (36例中)	EPP (203例中)	HEP (6例中)	PCT (328例中)	計 (573例中)
ステロイド	0	3	1	6	10
β -カロテン	1	18	0	3	22
ビタミン(主としてE)	1	1	0	21	23
皮膚外用薬(ステロイド)	16	6	0	25	47
瀉血	0	0	0	64	64
クロロキン	0	0	0	4	4
Ca-EDTA	0	0	0	11	11
インターフェロン	0	0	0	16	16
シメチジン	0	3	0	4	7
肝庇護剤	0	0	0	2	2
ヒトヘミン	0	1	0	0	1
PCG	0	2	0	0	2
その他(ノボリンR、ウルソ)	0	0	0	1	1

急性ポルフィリン症の治療薬を表 14 に示した。本症では共通してグルコースを主体とする大量の補液が治療の中心となり、疼痛のコントロールにはクロルプロマジンが用いられている[38]。最近では急性発作に対してシメチジンの投与[39]に関する報告が 13 例あり、そのうち 9 例に症状の改善がみられている。また、国外ではヘム・アルギニン酸、ヘマチン静注療法が比較的原因療法に近いものとして利用されているが[40]、本剤は国内では市販されていない関係から（2012 年度末よりヘム・アルギニン酸は保険適用される予定である）、報告例数は少ない[41,42]。

表14 急性ポルフィリン症の主たる治療薬

	AIP (198例中)	VP (56例中)	HCP (41例中)	分類不明 (58例中)	計 (353例中)
補液(+グルコース)	82	27	26	19	154
クロルプロマジン	59	15	19	12	105
AMP, ATP	14	3	3	0	20
ステロイド	28	4	2	4	38
ビタミン(主としてB群)	22	1	0	4	27
ACTH	8	0	0	0	8
ワゴスティグミン	6	0	0	0	6
Ca剤	5	0	0	0	5
テトクローム C	5	2	5	0	12
血漿交換	1	0	0	2	3
シメチジン	10	1	1	1	13
ヘム・アルギニン酸, ヘマチン	2	1	1	1	5

（2）予後

ポルフィリン症の予後について表 15 に示した。ポルフィリン症の全病型に対して、誘発因子を除去することが発症を予防する上で、また治療・予後において重要である。

急性ポルフィリン症では早期発見、血漿交換、人工呼吸器などの普及により死亡する症例が減少し、軽快する例が多いが、いまだに死の転帰をとるものの割合も高い。一方、PCT では不変、または増悪する例の割合が高く、C型肝炎との合併率が高いことなどから、今後肝癌死亡例が増加することが予想される。HEP では予後が悪く 6 例中 4 例が死の転帰（主に肝不全）をとっている。EPP では血中 PP 値が高い例ほど肝障害を起こす可能性が高く、肝硬変、肝不全により予後は悪い。

表15 ポルフィリン症の予後

	軽快	不変～悪化	死亡	不明数	剖検数
CEP	7	22	4	4	1
EPP	46	21	18	117	7
HEP	1	0	4	1	0
小 計	54	43	26	122	8
AIP	113	8	47	27	27
VP	34	6	9	7	4
HCP	32	2	1	6	0
分類不明	27	9	10	10	3
小 計	206	25	67	50	34
PCT	128	96	5	102	4
計	388	164	98	274	46

Ⅲ. 謝　辞

本研究は厚生労働科学研究費補助金（難治性疾患克服研究事業）「遺伝性ポルフィリン症の全国疫学調査並びに診断・治療法の開発に関する研究」およびその他の研究予算によって行われた。

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