

CASE REPORT

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An infantile case of hereditary folate malabsorption with sudden development of pulmonary hemorrhage: a case report

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Abstract

Background: Hereditary folate malabsorption—a rare disorder caused by impairment of the folate transporter—can develop into severe folate deficiency manifesting as megaloblastic anemia and occasionally thrombocytopenia. Reportedly, megaloblastic anemia can manifest with hemorrhagic episodes, possibly due to ineffective platelet production and platelet dysfunction. However, life-threatening hemorrhage events in hereditary folate malabsorption have not been well investigated.

Case presentation: A 3-month-old Japanese boy was transferred to our hospital due to thrombocytopenia and severe megaloblastic anemia. During a thorough examination of hematopoietic abnormalities, the patient suddenly went into cardiac arrest due to pulmonary hemorrhage. Although intravenous folate supplementation was started soon after the identification of folate deficiency, the patient died of circulatory defect and multiple organ failure. The cause of pulmonary hemorrhage, such as respiratory infection, could not be confirmed. Genetic investigation revealed a mutation in the *SLC46A1* gene to be the cause of the hereditary folate malabsorption.

Conclusion: We report an infantile case of hereditary folate malabsorption that progressed to lethal pulmonary hemorrhage before folate deficiency was identified. Clinicians should consider that megaloblastic anemia could lead to severe bleeding without warning, and that nutrient supplementation should be initiated as soon as possible.

Keywords: Case report, Hereditary folate malabsorption, Homocysteine, Megaloblastic anemia, *SLC46A1*

Background

Megaloblastic anemia is characterized by ineffective hematopoiesis, which frequently manifests as decreased mature healthy red blood cells and unusually large, abnormal, and immature erythrocytes that fail to enter blood circulation due to their large size [1]. Leukopenia or thrombocytopenia is also present [1]. The abnormal erythrocytes called megaloblasts are fragile, resulting in

intramedullary and extravascular hemolysis. The most frequent causes of megaloblastic anemia are deficiencies of either vitamin B12 or folate, both of which are cofactors required for DNA synthesis [2]. When DNA synthesis is impaired, the cell cycle cannot progress from the G2 growth stage to the mitosis (M) stage. This leads to cell growth without division, which manifests as unusually large, abnormal, and poorly developed erythrocytes (megaloblasts). In severe cases, thrombocytopenia, leukopenia, and hypersegmented neutrophils may be present, resulting from impaired nuclear differentiation [1].

Folate deficiency occurs due to poor dietary intake, exposure to antifolate drugs, and impairments in folate metabolism and transporters [2]. Hereditary folate

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malabsorption (HFM) is a rare autosomal recessive disorder caused by loss-of-function mutations in *SLC46A1*, the gene coding for the proton-coupled folate transporter (PCFT), which is an essential molecule for intestinal folate absorption and folate transport into the central nervous system [3]. Infants with this condition are normal at birth because of nourishment from the mother during fetal development. However, within a few months, they develop the symptoms of folate deficiency, such as anemia, immunoglobulin deficiency, infections, diarrhea, and later, neurological damage.

We present a case of HFM in a 3-month-old Japanese boy, which manifested with megaloblastic anemia and thrombocytopenia. Laboratory examination showed severe folate deficiency, and genetic analysis revealed the presence of a deep intronic mutation (c.1166-285 T>G of *PCFT-SLC46A1*), resulting in a 168-bp insertion in cDNA [4]. It was not until serum folate was found to be undetectable that the patient developed sudden pulmonary hemorrhage. Although life-threatening hemorrhage events in previously reported HFM cases have been rare, this case warns us to pay attention to the bleeding tendency of this disease.

Case presentation

Patient history

A 3-month-old Japanese baby was transferred to our institute due to severe anemia and thrombocytopenia.

He was the first child of healthy, non-consanguineous parents with no family history of hematological or congenital disorders. During the first month of his life, he gained weight exclusively from breastfeeding; however, he gradually started vomiting and having diarrhea. Laboratory examinations revealed severe anemia (hemoglobin, 52 g/L) and thrombocytopenia (platelet count, $37 \times 10^9/L$) with the presence of schistocytes (8% of red blood cells). Mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration were 84.4 fL, 30 pg, and 360 g/L, respectively. Reticulocyte elevation was inadequate (28% of red blood cells). Although lactate dehydrogenase was elevated to 1728 U/L, and total bilirubin and potassium were also elevated, direct and indirect Coombs tests were negative (Table 1).

Bone marrow aspiration showed hyperplasia and megaloblastic change of erythroblasts, both of which are indicative of megaloblastic anemia. Dysplasia of granulocytes was also confirmed, and the percentage of megakaryocytes was decreased (Fig. 1). While further examinations were being performed, the patient suddenly developed pulmonary hemorrhage, resulting in cardiopulmonary arrest. Although the patient responded to cardiopulmonary resuscitation, he developed severe hypoxic-ischemic encephalopathy, respiratory insufficiency, and multiple organ dysfunction syndrome. Subsequently, he was started on intensive care including

Table 1 Results of laboratory examination at diagnosis

WBC	$6.01 \times 10^9/L$	PTINR	1.08	TP	53 g/L	Ferr	420 $\mu g/L$
Neut	$0.84 \times 10^9/L$	APTT	34.5 seconds	ALB	41 g/L	Fe	51 $\mu mol/L$
Lymp	$4.99 \times 10^9/L$	Fib	0.8 g/L	T-Bil	58 $\mu mol/L$	UIBC	4.7 $\mu mol/L$
		D-d	5340 $\mu g/L$	D-Bil	5.1 $\mu mol/L$	TIBC	56 $\mu mol/L$
RBC	$1.73 \times 10^{12}/L$	ATIII	76%	AST	70 U/L		
Hb	52 g/L			ALT	34 U/L	Folate	< 0.9 nmol/L
MCV	84.4 fL			LDH	1728 U/L	Vit.B12	31 pmol/L
MCH	30 pg	C3	0.6 g/L	BUN	3.9 mmol/L	Hp	< 0.10 g/L
MCHC	360 g/L	C4	0.2 g/L	Cre	22 $\mu mol/L$	Direct Coombs	(-)
Ht	0.146 fraction	CH50	5740 U/L	Na	138 mmol/L	Indirect Coombs	(-)
Plt	$37 \times 10^9/L$			K	5.0 mmol/L	O157 LPS Ab	(-)
Ret	$4.8 \times 10^9/L$	IgG	2.58 g/L	Cl	105 mmol/L	ADAMTS13	0.989 IU/mL
Schistocyte	$138 \times 10^9/L$	IgA	0.09 g/L	Ca	2.32 mmol/L		
		IgM	0.09 g/L	IP	1.32 mmol/L		
				CRP	< 1.0 mg/L		

WBC white blood cell, Neut neutrophil, lymp lymphocyte, RBC red blood cell, Hb hemoglobin, MCV mean corpuscular volume, MCH mean corpuscular hemoglobin, MCHC mean corpuscular hemoglobin concentration, Ht hematocrit, Plt platelet, Ret reticulocyte, PTINR international normalized ratio of prothrombin time, APTT activated partial thromboplastin time, Fib fibrinogen, D-d D-dimer, ATIII antithrombin, C3 complement component 3, C4 complement component 4, CH50 50% hemolytic unit of complement, IgG immunoglobulin G, IgA immunoglobulin A, IgM immunoglobulin M, TP total protein, ALB albumin, T-Bil total bilirubin, D-Bil direct bilirubin, AST aspartate aminotransferase, ALT alanine aminotransferase, LDH lactate dehydrogenase, BUN blood urea nitrogen, Cre creatinine, Na sodium, K potassium, Cl chloride, Ca calcium, IP inorganic phosphorus, CRP C-reactive protein, Ferr ferritin, Fe ferrum, UIBC unsaturated iron binding capacity, TIBC total iron binding capacity, Vit.B12 Vitamin B12, Hp haptoglobin, O157 LPS Ab O157 lipopolysaccharide antibody, ADAMTS13 a disintegrin-like and metalloproteinase with thrombospondin type 1 motifs 13

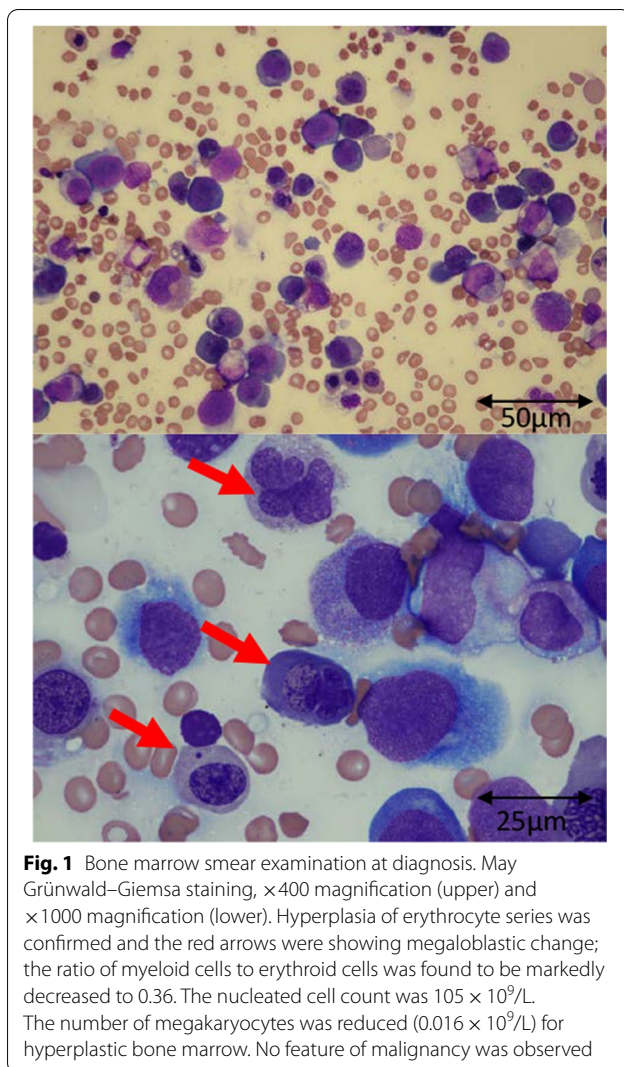


Fig. 1 Bone marrow smear examination at diagnosis. May Grünwald–Giemsa staining, $\times 400$ magnification (upper) and $\times 1000$ magnification (lower). Hyperplasia of erythrocyte series was confirmed and the red arrows were showing megaloblastic change; the ratio of myeloid cells to erythroid cells was found to be markedly decreased to 0.36. The nucleated cell count was $105 \times 10^9/L$. The number of megakaryocytes was reduced ($0.016 \times 10^9/L$) for hyperplastic bone marrow. No feature of malignancy was observed

hemodialysis, plasma exchange, extracorporeal membrane oxygenation, and frequent blood transfusions. On the fifth day of hospitalization, serum folate level was found to be undetectable (normal, 11–34 nmol/L). This led us to confirm that folate deficiency was the cause of the megaloblastic anemia. Although intravenous folate supplementation (folinic acid, 6 mg/day) was initiated, the patient died of circulatory failure 19 days after hospitalization (Fig. 2). Examination of autopsy samples revealed that the megaloblastic change in his bone marrow had disappeared; however, the cause of pulmonary hemorrhage could not be confirmed. An amino acid analysis conducted before intravenous folate replenishment indicated high levels of serum homocysteine, serum cysteine, and urine cysteine, and a low level of serum methionine, which normalized after the administration of medication (Table 2). In Japan, all newborns

are screened for metabolic disorders. On reanalysis of his neonatal blood sample, methionine, homocysteine, and cysteine levels were found to be normal (Table 2). Thus, the abnormal pattern of amino acids was consistent with HFM.

Genetic analysis

This study was approved by the ethics committee of Asahikawa Medical University (no. 18231). Blood samples were collected from the patient and his parents after obtaining their informed consent. DNA was isolated using the Genra Puregene blood kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. RNA was isolated from the father's blood sample and used to synthesize complementary DNA (cDNA) by employing the SuperScript III first-strand synthesis system (Invitrogen, Carlsbad, CA, USA) for reverse-transcription polymerase chain reaction (RT-PCR). Direct sequence analyses targeting *SLC46A1* exons 1–5 and intron 3 were performed according to previous reports [3, 4]. The following primers were used in the process: exon1-forward, 5'-CGCCGGACATTTAAG GAG-3'; exon1-reverse, 5'-ATGGTAGTGGCGGGT AACTG-3'; exon2(anterior half)-forward, 5'-AGGTTT AGGGCTCCAAAGGA-3'; exon2(anterior half)-reverse, 5'-TAAAGTGTGTGGGCTCAGGG-3'; exon2(posterior half)-forward, 5'-ATGCTGGCAAGCCTCCTC-3'; exon2(posterior half)-reverse, 5'-GAAATCCCTCAA AAATGCCA-3'; exon3-forward, 5'-TAGGAGCTCTGC TGCCTTTC-3'; exon3-reverse, 5'-TGTCTGGTTTCT CATGTCCA-3'; exon4-forward, 5'-GAATATGGCCCT TTCGGACT-3'; exon4-reverse, 5'-TGAACCCATGCT CATAATGG-3'; exon5-forward, 5'-AGGAGGAGGTTT CAGGAGAG-3'; exon5-reverse, 5'-GCAAAGTGAACA CCAAGCAA-3'; intron3-forward, 5'-AGCAGAAGG AGGAACAGGAA-3'; intron3-reverse, 5'-TCCACATAT GACCTTACTGAATCC-3'.

Although no significant mutations were detected in the exon sequences of the patient, a homozygous mutation of c.1166-285 T>G was detected (Fig. 3) in intron 3. His parents were heterozygous for the mutation.

Discussion and conclusions

Our patient was doing well, and folate deficiency was not suggested until he turned 1 month old. Folate and vitamin B12 play key roles in the methionine cycle to remethylate homocysteine to methionine, and the serum homocysteine level is elevated when there is a shortage of folate or vitamin B12 [2]. Based on this, it is possible that the patient was not deficient in folate at birth, because serum homocysteine at birth was not elevated (Table 2). In the subsequent few months, folate deficiency must

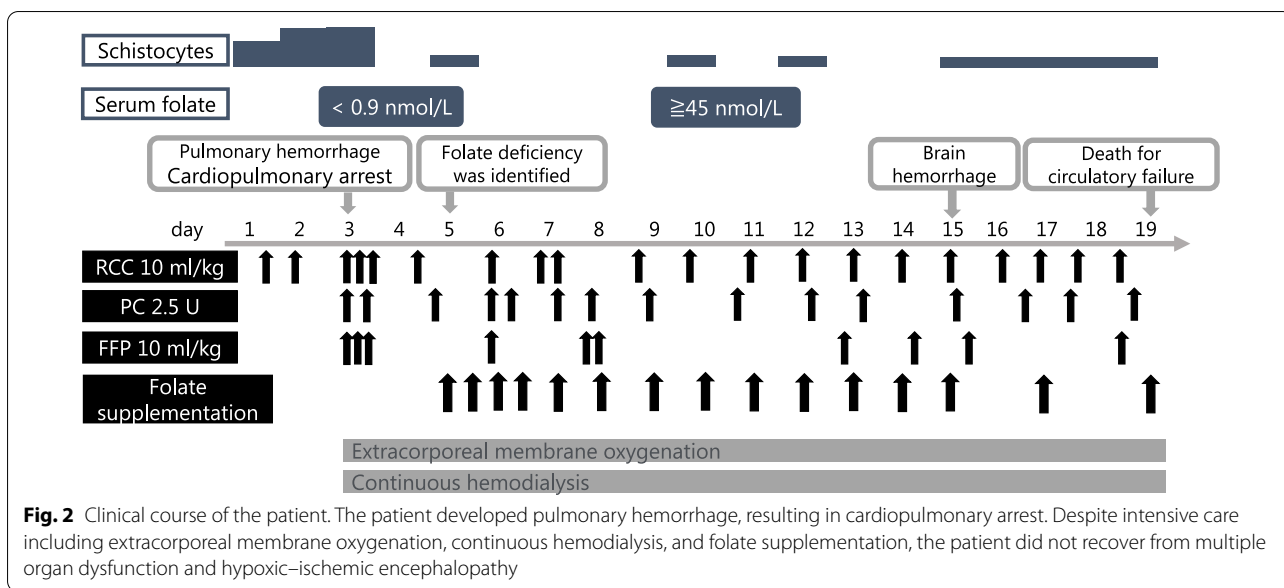


Fig. 2 Clinical course of the patient. The patient developed pulmonary hemorrhage, resulting in cardiopulmonary arrest. Despite intensive care including extracorporeal membrane oxygenation, continuous hemodialysis, and folate supplementation, the patient did not recover from multiple organ dysfunction and hypoxic-ischemic encephalopathy

Table 2 Clinical variation of plasma amino acids associated with folate supplementation

	Normal value	At birth	Admission	After supplementation
Serum folate	11–34 (nmol/L)	N.A.	< 0.9	≥ 45
Serum methionine	18.9–40.5 (μmol/L)	24.014	8.8	29.7
Serum homocysteine	1.8–4.6 (μmol/L)	1.6	21.0	N.D.
Serum cysteine	13.7–28.3 (μmol/L)	22.9	32.4	15.9

N.A. not available, N.D. not detected

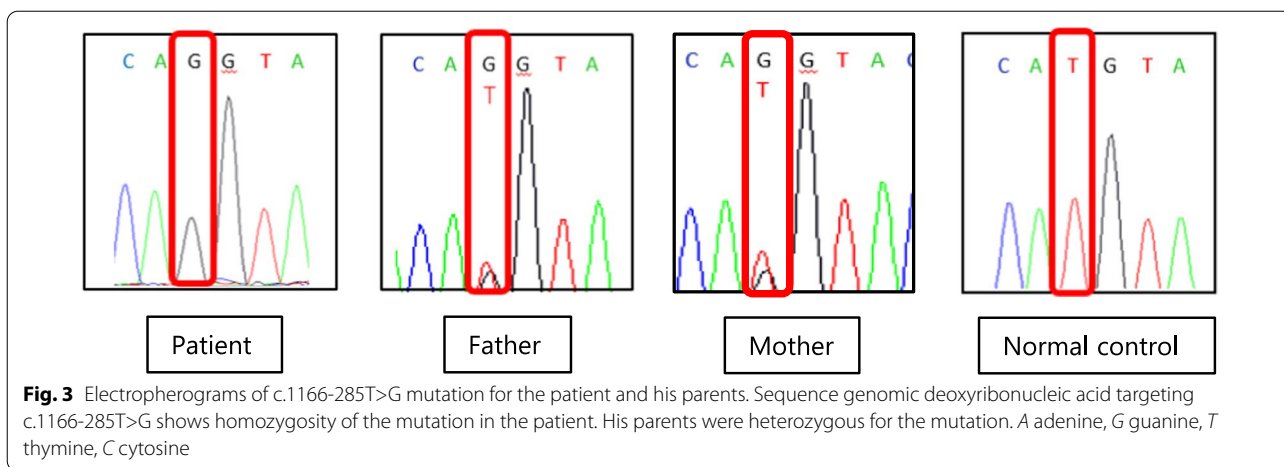


Fig. 3 Electropherograms of c.1166-285T>G mutation for the patient and his parents. Sequence genomic deoxyribonucleic acid targeting c.1166-285T>G shows homozygosity of the mutation in the patient. His parents were heterozygous for the mutation. A adenine, G guanine, T thymine, C cytosine

have progressed due to impaired absorption through the intestine, resulting in the manifestation of megaloblastic anemia and thrombocytopenia. On admission to our hospital, the serum folate level was reduced to undetectable levels. On the contrary, the serum homocysteine level was found to be increased. The appearance

of schistocytes in the peripheral blood may indicate the presence of dyserythropoiesis in case of folate deficiency [5].

Gene analysis revealed a c.1166-285 T>G mutation in intron 3 of *SLC46A1*. This replacement is reported to induce splicing changes and cause a 168-bp insertion

between exons 3 and 4, leading to premature termination and development of HFM [4]. Both parents were heterozygous for this mutation.

Since Qiu *et al.* demonstrated the molecular basis for HFM [6], 36 cases of genetically confirmed HFM have been reported [7, 8]. HFM is generally diagnosed in early infancy; most patients develop recurrent infection, failure to thrive, or developmental delays from 2 months to 1 year of age. Megaloblastic anemia was present at onset in all cases, except in those who received early folate supplementation due to family history. Seizures tended to manifest in cases in which folate supplementation was delayed or started orally. Serum folate levels before supplementation were undetectable in more than half of cases.

Although only a few bleeding episodes have been reported in previous HFM cases, it should be noted that our patient exhibited a sudden and serious course of pulmonary hemorrhage. The bleeding occurred while preparing for a transfusion with a platelet count of $13 \times 10^9/L$. Another patient with HFM developed alveolar hemorrhage concomitant with thrombocytopenia ($18 \times 10^9/L$) [4]. In addition, intracranial hemorrhage was reported in PCFT-deficient mice [9]. Judging from these facts, it is important to recognize bleeding tendency as one of the features of this disease.

Considering that prophylactic platelet transfusion is generally recommended at a platelet count equal to or less than $10 \times 10^9/\mu L$ in a variety of conditions involving thrombocytopenia [10], it cannot be simply assumed that a low platelet count was the cause of pulmonary hemorrhage. There might have been additional factors contributing to this event.

Hemorrhagic events such as retinal hemorrhages and purpuric spots have been reported in patients with megaloblastic anemia caused by vitamin B12 deficiency. Bleeding in the form of hematemesis, hemoptysis, and even life-threatening bleeding have also been reported [11]. Interestingly, Saloujin *et al.* reported that, in cases of megaloblastic anemia caused by vitamin B12 deficiency, the function of platelets is deteriorated [12]. Therefore, it is possible that platelet function is deteriorated in conditions of HFM. This might have been the factor that made the bleeding tendency much more severe than we speculated based on the platelet count, although we were unable to perform platelet function tests due to the severe clinical course.

Elevated serum homocysteine might cause endothelial dysfunction [13]. If hyperhomocysteinemia is severe (around $100 \mu mol/L$), thromboembolism is often combined with endothelial damage, which may lead to thrombotic microangiopathy and diffuse alveolar hemorrhage, as previously reported in vitamin B12 disorder

[14–16]. However, our case presented only mild elevation of homocysteine ($21.0 \mu mol/L$; normal range $1.8–4.6 \mu mol/L$). This is within the recommended level (below $30 \mu mol/L$) [17]. Therefore, a contribution of the elevated homocysteine to the bleeding tendency can be excluded.

We describe herein a pediatric case of megaloblastic anemia with HFM complicated by severe pulmonary hemorrhage. Thrombocytopenia and possible platelet dysfunction were considered to be the main causes. Life-threatening hemorrhage has not been well documented in HFM accompanied by megaloblastic anemia, possibly because of the rarity of HFM. However, it is undoubtedly an important complication of megaloblastic anemia. When an infant presents with megaloblastic anemia, folate or vitamin B12 deficiency should be assessed immediately, and supplementation of these elements should be initiated as soon as possible, even before the cause of megaloblastic anemia is identified.

Abbreviations

HFM: Hereditary folate malabsorption; PCFT: Proton-coupled folate transporter.

Acknowledgements

Not applicable.

Author contributions

NT contributed to the conception and design of the genetic analysis; HK and MN performed the pathological evaluation of autopsy samples; TI introduced the patient to our institute. TS and HA critically reviewed the manuscript and supervised the entire study process. All authors read and approved the final manuscript.

Funding

There are no sources of funding directly relevant to the content of this article.

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

This issue was approved by the ethic committee of Asahikawa Medical University (no. 18231).

Consent for publication

Written informed consent was obtained from the patient's legal guardian for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Competing interests

The authors declare that they have no competing interests.

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Received: 11 February 2022 Accepted: 6 May 2022

Published online: 30 June 2022

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