

Studies on Familial Hypotransferrinemia: Unique Clinical Course and Molecular Pathology

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Summary

Some unsolved problems—late onset of anemia and growth retardation (at age 7 years), healthy siblings showing very low transferrin (TF) level, and unexplained mode of inheritance—were found in family members of a congenital atransferrinemia already reported in 1972. The long-term clinical, laboratory, and developmental observations revealed that after 5 years of apo-TF supplementary therapy the patient's anemia gradually disappeared, and he started to grow again without further therapy. Immunoelectrophoretic study disclosed a severe deficiency of both TF and haptoglobin in the patient. The recovery from his anemia and the resumption of his physical development were dependent only on his TF level: that is, from a negligible level it increased to 10–20 mg/dl (normal, 205–370 mg/dl), a level similar to that of his TF-deficient siblings, who had been in good health since birth. The TF analysis of the patient and his family suggests that the minimum TF requisite in this family may be close to 10–20 mg/dl; subjects with more than 20 mg/dl are apparently healthy; with less than 10 mg/dl they may develop severe growth retardation and anemia, and extreme deficiency may be lethal. Also, coexisting haptoglobin deficiency might alleviate hemosiderosis. Further, the isoelectric focusing study disclosed that there was only a small amount of TF variant in these siblings including the patient. The study of the family confirmed that this variant was produced by an allelic gene derived from their father. So, the original diagnosis of congenital atransferrinemia should be revised as familial hypotransferrinemia transmitted with autosomal recessive mode, and the subjects with a recessive character may be compound heterozygotes of the “variant” allele and the “null” allele.

Introduction

Iron is essential for life in all organisms. Therefore it ought to evolve specific molecules in order to maintain itself in a soluble form for transport, storage, and utilization. In vertebrates, serum transferrin (TF) is, as an iron-transport protein, responsible for conveying ferric iron between the sites of absorption, storage, and utilization (Seligman et al. 1987).

Iron metabolism disorder determined by single gene mutations provides new information on the mechanism

and control of iron transport. Some extreme cases of such mutations suggest that less dramatic variations in genetic control of iron metabolism may have important long-term effects on an organism. Among very rare human disorders, congenital atransferrinemia is of great physiological interest (Fairbanks and Beutler 1990). The first patient reported was a girl of 3 mo who had profound hypochromic anemia resistant to most treatments. When she was age 7 years, investigations by Heilmeyer et al. (1961) disclosed that the patient's plasma contained only a trace of TF. Since the first patient, only several cases of hereditary atransferrinemia have been reported (Cáp et al. 1968; Sakata 1969; Walbaum 1971; Loperena et al. 1974; Hamill et al. 1991), but these reports have been far from being satisfactory from the viewpoint of modern medical science.

The case in the present paper, originally reported by Goya et al. (1972) as congenital atransferrinemia, has

Received January 27, 1993; revision received March 16, 1993.

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0002-9297/93/5301-0024\$02.00

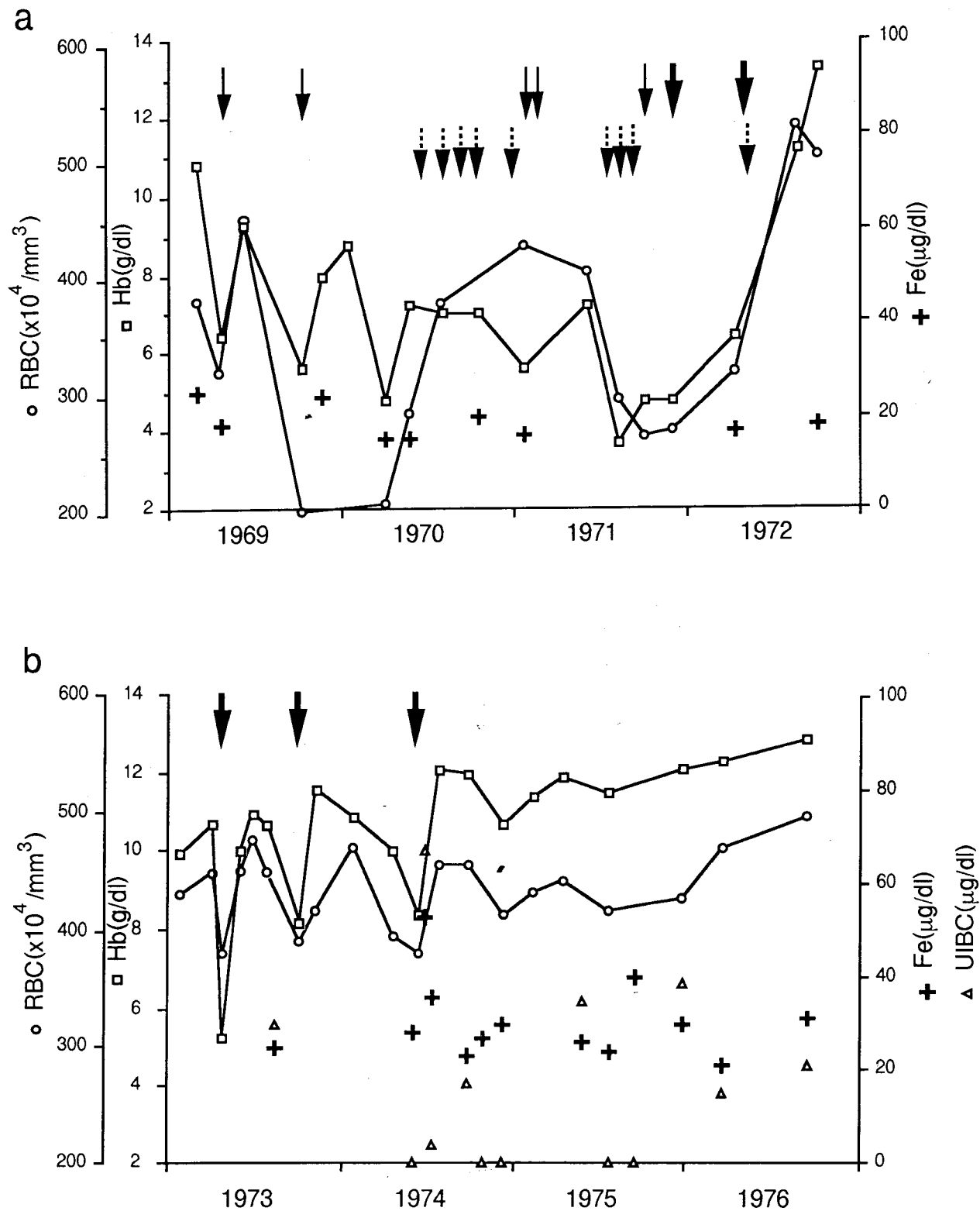


Figure 1 Clinical course of the hypotransferrinemic patient at Kyushu University Hospital (a) and at Osaka University Hospital (b). At Kyushu University Hospital, the patient was treated with infusion of desferri-apo-TF, 1.0 g (indicated by the smaller arrows with unbroken shafts) or 2.0 g (indicated by the larger arrows), and with transfusion of whole blood, 200 ml (indicated by the smaller arrows with broken shafts). On the basis of this result, infusion of only desferri-apo-TF was continued at Osaka University Hospital. The effect of treatment was monitored by red blood cell count (RBC; \circ) and hemoglobin concentration (Hb; \square), as markers for anemia, and by serum iron level (Fe; $+$) and UIBC (\triangle), as markers for iron metabolism.

presented some questions in the course of our long-term clinical observation:

1. Though the condition was considered as hereditary, the clinical signs first appeared at the age of 7 years; since birth, the patient had shown no signs of anemia or growth retardation.
2. Some of his healthy siblings showed remarkable decrease of TF in serum ($1/20$ – $1/10$ of normal value), but none of them had any pathological symptoms.
3. The analyses of serum TF of his family clearly showed that it was a genetic disease, but none of the types of Mendelian inheritance has matched this type in question.
4. What is the molecular basis for the patient and his healthy siblings showing decreased TF level?

In the present paper, the authors give answers to the questions above, through the long-term clinical observation of the patient and the electrophoretic study of serum TF on protein level.

Subjects, Material, and Methods

Case History

In 1960 the patient was born as a small-birthweight baby (2,440 g at 40 wk), and he grew up in good health. At the age of 7 years, he had a cold and complained of a high fever (40°C) and a sore throat. A physician gave him chloramphenicol (1.0 g orally daily, for several days), under the diagnosis of acute tonsillitis. Three months after the febrile episode, he complained of a sudden fainting attack and was introduced to N. Goya at Kyushu University Hospital as a suspected case of heart failure. After 3 mo of careful examination, he was diagnosed as a case of congenital atransferrinemia. After that, he was treated with intermittent blood transfusion and apo-TF infusion.

In 1973, the proband was first seen by A.H. at Osaka University Hospital, at the request of N. Goya, for the follow-up treatment. The physical examination revealed a short and emaciated state but no signs of anemia, jaundice, or heart and lung failure. Although neither motion and sensitivity disturbances nor abnormal tendon reflex was observed, physical and verbal responses were very slow and were accompanied by occasional involuntary movements such as a tic. On laboratory examination, the patient was found to have no hematological abnormalities but some disturbances in the liver function and in electroencephalogram. The most remarkable finding was that regarding serum TF content; only a trace was present in the patient, about

$1/20$ of normal value in his older brother and younger sister, about half in both of the parents, and about two-thirds in his younger brother. These findings partially supported the data, especially those of serum TF level, collected by Kyushu University Hospital.

The patient and his family members were those already reported by Goya et al. (1972). Informed consent was obtained from all family members.

Material and Methods

Desferri-apo-TF used in the supplementary therapy was supplied by the courtesy of Dr. H. G. Schwick (Behringwerke AG, Germany).

Laboratory examination.—Hematologic data were obtained by conventional methods. Serum iron and total iron-binding capacity (TIBC) were determined by colorimetry before 1982 and by an electrochemical technique after that. Serum TF (before 1975) and hemopexin were determined by using a Nor-Partigen plate (Hoechst) in the manner of Mancini et al. (1965). TF (after 1977), haptoglobin, ceruloplasmin, and α_1 -antitrypsin were determined by using the turbidimetric immunoassay of Ritchie (1975); ferritin by using radioimmunoassay of Reeves and Haurani (1980); and bilirubin by using the alkaline azo bilirubin method of Jendrassik and Grof (1938).

Isoelectric focusing and immunoblotting.—Prior to isoelectric focusing, the serum samples from both parents, the younger brother, and normal controls were diluted 1:4, and those from the patient, his older brother, and his sister, all with very low TF levels, were diluted 1:1 with 0.3% ferrous ammonium sulfate solution. Then the mixtures were incubated at 37°C for 3 h. Isoelectric focusing was carried out on polyacrylamide slab gel containing Ampholine pH5-7 (Pharmacia LKB, Bromma, Sweden) according to a method described by Pascali et al. (1982). The running conditions were slightly modified; prefocusing was carried out at 10 W for 1 h, and focusing was carried out at 20 W for 1.5 h on a gel of dimensions 115 × 230 × 1 mm. After the focusing, the proteins were transferred electrophoretically onto a nitrocellulose sheet. Finally, TF bands were specifically detected by an antibody reaction using both polyclonal anti-human transferrin conjugated with peroxidase (The Binding Site, Birmingham, England) and, as substrate for color reaction, 4-chloro-1-naphthol.

Desialylation and isoelectric focusing.—Desialylation of TF was performed by adding 10 ml (0.1 unit) of neuraminidase from *arthrobacter ureafaciens* (Boehringer Mannheim, Germany) to the mixture of serum

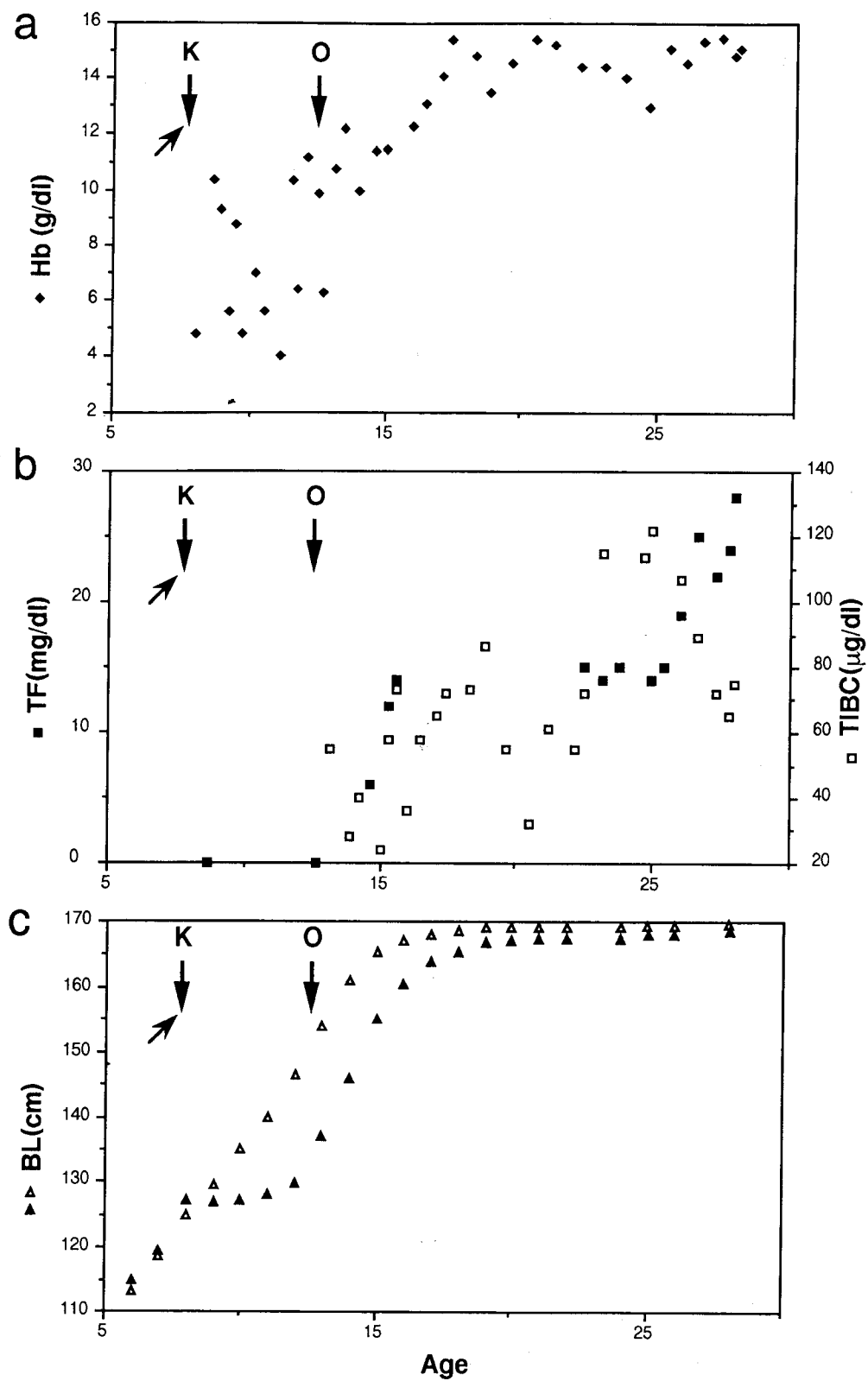


Figure 2 Hypotransferrinemic patient's course of anemia (a) and physical development (c), as associated with TF and iron metabolism (b). His clinical course was followed at Kyushu University Hospital (indicated by arrows with a "K") and Osaka University Hospital (indicated by

and sodium acetate solution (50 mM, pH 5.0), followed by incubation at 37°C for 18 h. Then, after iron resaturation, isoelectric focusing and immunoblotting were carried out as mentioned above.

Immunoelectrophoresis (Williams 1971).—After electrophoresis of serum on agar gel, double diffusion of the specific protein and its antiserum was performed. Then the precipitated protein bands were stained with amidoblack 10 B.

Results

Clinical Course of the Patient at Kyushu and Osaka University Hospitals

In the early stage of hospitalization at Kyushu University Hospital, as already reported, the effect of apo-TF supplementary therapy was examined and partially confirmed. However, since the details of the necessary dose or the intervals were not established, serious anemia often ensued, and whole-blood transfusion had to be performed, as shown in figure 1a. Throughout the clinical course, the serum iron level was always low, about $1/5$ of normal.

On the basis of both the patient's clinical course at Kyushu University and the laboratory data collected at Osaka University, only apo-TF supplementary therapy was continued—without blood transfusion, which had already been confirmed, by Heilmeyer (1966), to cause severe iron deposit and liver cirrhosis. When hemoglobin concentration reached the level of less than 10 g/dl, 2.0 g of desferri-apo-TF in 500 ml of physiological saline was infused. As shown in figure 1b, after the first apo-TF infusion at Osaka University Hospital, the level of hemoglobin concentration rapidly reached the normal level within 1 wk, and its effect was confirmed to continue for about 4–5 mo. After the second infusion, however, its effect was prolonged for about 9 mo, and finally, after the third infusion, the patient needed no more apo-TF supplementary therapy. During this clinical course, the serum iron level was as low as it had been at Kyushu University Hospital. However, the level of unsaturated iron-binding capacity (UIBC) was irregular: the level was often immeasurably low until 1975, but later it remained at 20–40 mg/dl, which is still very low.

Course of Anemia and Physical Development Associated with TF Level

Figure 2 shows the change in hemoglobin concentration, serum TF level or TIBC, and body length. The hemoglobin concentration as a marker of anemic state had fluctuated for several years, in spite of blood transfusion or apo-TF supplementary therapy. However, it began to stabilize around the age of 13 years and has been maintained at a normal level since the patient was 17 years old (fig. 2a). In relation to this change, a distinct increase of serum TF level was observed; though it had been present only in trace amounts for several years since the patient first suffered from severe anemia, it began to rise to a level that allows measurement, even though it was still very low, and finally it reached 14–30 mg/dl (about $1/20$ – $1/10$ of normal value). Also, TIBC changed almost in parallel with serum TF level, though there was a little dissociation at some stages (fig. 2b). Furthermore, as shown in figure 2c, a dramatic change occurred in the patient's body length, in concert with these alterations in hemoglobin and TF levels; the patient's physical and mental development had been within the normal range since birth, until he had some febrile attack and successive anemia at the age of 7 years. After these episodes, his development, especially that of his body length, was suddenly suspended, and the growth retardation continued for about 5 years. Then, he began to grow again around the age of 13 years, when apo-TF supplementary therapy was well under way, finally reaching, at about the age of 25 years, the mean body length for an average Japanese boy who is the same age and who was born in 1960 (Japanese Ministry of Health and Welfare 1987). The growth of his mental development was also remarkable; he had to attend a special class for the retarded in junior high school, but he went on to a class for normal students in senior high school and graduated from it, with excellent grades.

The present state of the patient and his TF-deficient siblings is as follows: the patient is working as a dental technician, and his older brother is working as a salesman, both without any pathological signs. The younger sister got married about 3 years ago and recently had a healthy baby girl, without any trouble throughout her pregnancy and delivery.

arrows with an "O"), after the onset of the patient's fainting attack (indicated by the diagonal arrows), and his physical development was followed from age 5 years (i.e., before the attack). The following indexes were employed: hemoglobin concentration (Hb; ◆), for anemia; serum TF level (TF; ■) or TIBC (□), for iron metabolism; and patient's body length (BL; ▲) compared with the mean body length of a Japanese boy of the same age and born in 1960 (BL; △), for physical development.