Hemoglobinopathy in Japan

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Hemoglobin is a chromoproteid consisting of heme and globin. The globin is an ellipsoid molecule composed of four polypeptides, namely α and β chains in pairs, in a manner as expressed by $\alpha_2\beta_2$ (Figure 1). Hemoglobin possessing α or β chain of abnormal amino acid sequence is referred to as abnormal hemoglobin.[1,2] There are a number of hereditary diseases in which synthesis of normal hemoglobin is partially or completely displaced by the production of abnormal hemoglobin. They are called hemoglobinopathies.[3] Sickle cell anemia and thalassemia are their typical example.

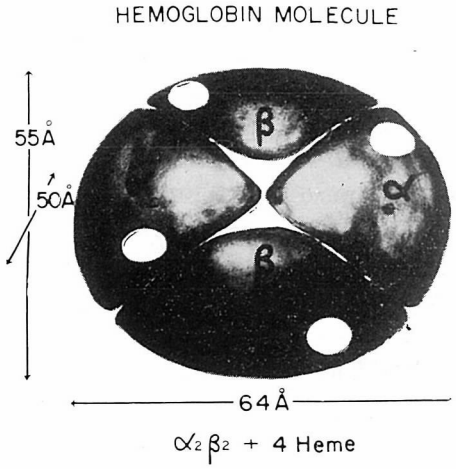

Figure 1. Hemoglobin molecule ($64 \times 55 \times 55$Å). The molecule is composed of α and β chains in pairs. The white disk in each chain refers to heme.

In the Far East, hemoglobinopathies are prevalent in the countries adjoining Japan. China is now regarded to be a cradle of thalassemia and thalassemia-Hb H disease. Thailand is well known for the endemicity of thalassemia-Hb E disease, and hemoglobin E is not uncommonly found among the people of Indonesia and the Philippines.[4] (Figure 2)

Present anthropology postulates that the ancestors of Japanese came to the four islands of Japan in ancient times either from the South Sea Islands or from the

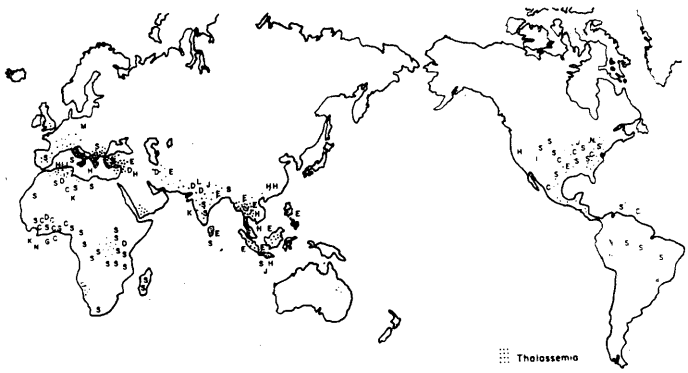

Figure 2. Geographical distribution of hemoglobinopathy in the world (Hemoglobinopathy in Japan is excluded from this map)

northern continent of the Far East. No matter which direction they came from, it is reasonable to assume that they had brought and handed down to their descendants some abnormal hemoglobin primarily of the land where they originated. There is also a possibility that hemoglobins S and hemoglobin C, the abnormal hemoglobins among negroes, may be found in the mixed-blood children born to Japanese mother and American Negro fathers who were in military service and came to Japan after the end of the Pacific War.

Nevertheless, hemoglobinopathy was not a subject of interest for the hematologists in Japan a few years ago, because the concensus of their conception was that Japanese people seemed to be exempted from this disease.

A systematic survey of hemoglobinopathy in Japan dates back to 1957, when several hematologists began the study with the conventional techniques (paper electrophoresis, solubility test, alkali denaturation test, etc.) in Tokyo, Gifu, Kyoto, Ube and Fukuoka. The author who established a survey center in Ube, Yamaguchi Prefecture, examined approximately 1700 Japanese people during the peiod from 1957 to the end of 1959.' Our servey failed to detect even a single case of hemoglobinopathy, only to support the then predominant view among the Japanese hematologist who vaguely anticipated its absence in this country.[6]

In spite of negative results, the surveys were carried on. The reward came shortly after the end of 1959, when several abnormal hemoglobins were reported all at once from different parts of this country. Early in 1960 an abnormal hemoglobin with chocolate brown color was isolated in the author's laboratory by means of agar gel electrophoresis from a blood sample of a patient with hereditary nigremia (Tamura-Takahashi's disease)[5] which was endemic in Iwate Prefecture. This was the first abnormal hemoglobin ever discovered in this country.[7] It was designated Hb M_I, and later, Hb M_{Iwate}.

At the present time (1963) it is estimated that approximately 30,000 people of

purely Japanese ancestry have received the examination of hemoglobin study all over this country and thirteen abnormal hemoglobins have been demonstrated in their blood. The frequency of hemoglobinopathy in this country is appraised to be one per 2000 to 3000.

The majority of the abnormal hemoglobins were detected exclusively from the members of the families of a single pedigree, but some of them (Hb Hikari and Hb Kokura) were found in two independent families which were not related mutually by marriage. It is therefore thought that there are a variety of abnormal hemoglobins in Japan, although they are rarely encountered.

Abnormal hemoglobins in Japan

The abnormal hemoglobins in Japan are classified into the following three groups.

1) Fast-moving hemoglobins which electrophoretically migrate to anode faster than Hb A (normal adult hemoglobin) at pH 8.6:—Hb Hikari ($\alpha_2^A\beta_2^{61\,Asp\,NH_2}$),[8] Hb Tsukiji ($\alpha_2^A\beta_2^{Tsukiji}$),[9] Hb Ube-2 ($\alpha_2^{Tp\text{-}9}\beta_2^A$)[6] and Hb Tokuchi ($\alpha_2^A\beta_2^{2\,Tyr}$).[10]

2) Hb M which is chocolate brown in color and electrophoretically difficult to separate from Hb A:—Hb M_{Iwate} ($\alpha_2^{87\,Tyr}\beta_2^A$) and Hb M_{Kurume} ($\alpha_2^A\beta_2^{63\,Tyr}$).

3) Slow-moving hemoglobins which are slower in anodic migration than Hb A at pH 8.6:—Hb Tokyo ($\alpha_2^A\beta_2^{Tokyo}$), Hb Yukuhashi,[11] Hb Fukuoka,[11] Hb Kokura ($\alpha_2^{Kokura}\beta_2^A$),[11] Hb Shimonoseki[11] ($\alpha_2^{54\,Arg}\beta_2^A$), Hb Matsue ($\alpha_2^{Tp\text{-}9}\beta_2^A$)[12] and Hb Ube-1 ($\alpha_2^A\beta_2^{93\,Cys(sx)}$).

Most of these hemoglobins are not directly concerned with the causation of manifest clinical symptoms. However, hemoglobin M's, Hb Tokyo and Hb Ube-1 are the exceptions. Hb M disease is characterized by cyanosis, while Hb Tokyo and Hb Ube-1 diseases are hemolytic anemias associated with splenomegaly.

Hemoglobinopathies producing hemolytic anemia

Under this heading are grouped Hb Tokyo disease, Hb Ube-1 disease and thalassemia minor.

1) Hb Tokyo disease.[13] A pedigree of mild hemolytic anemia with the production of a slow-moving hemoglobin was discovered by Fukutake and Kato in Tokyo in 1960. This hemoglobin migrated between Hb A and Hb F on paper electrophoresis (pH 8.6). Solubility and alkali denaturation of the hemolysate was normal. Beta-chain anomaly was suspected for this hemoglobin from the result of acid-dissociation (starch block) electrophoresis and fingerprinting.

2) Hb Ube-1 disease.[14] This concerns with a fifteen years old girl. At the age of 7 the patient underwent medical treatment for the first time with the complaint of high fever in a hospital in Ube, where she was told that she had splenomegaly. Three years later (at the age of 10) she returned to the same clinic because she had

pallor of the face and yellow discoloration of sclera bulbi. A firm spleen was palpable 2.5 finger breadths below the left costal margin. A diagnosis of hypersplenism was entertained, and she was splenectomized. Following the splenectomy there was a considerable alleviation of anemia, but she remained slightly icteric. At the age of 13 her blood sample was sent to the department of clinical pathology, Yamaguchi Medical college, where a slow-moving hemoglobin equaling to Hb S in electrophoretic migration was demonstrated. The patient soon received the conventional hematological study of the peripheral blood, which revealed numerous erythrocytes containing a single Heinz body. There was a considerable increase in reticulocytes. Urine was distinctly positive for urobilinogen.

The abnormal hemoglobin was purified by starch block electrophoresis for the examination of chain anomaly (hybridization with canine hemoglobin) and fingerprinting. Chromatography (Amberlite IRC 50 and carboxymethyl cellulose) was also performed. These tests disclosed that the abnormal hemoglobin was susceptible to degeneration producing insoluble pigment and it had β chain anomaly, whereas there was no abnormality in fingerprinting. The hemoglobin was examined also for the core with negative abnormality. However, titration of active SH radicals with p-chloromercuribenzoic acid disclosed that this hemoglobin had an active SH-groups on account of the blockage of cysteine (93 rd amino acid residue) of its β chains.

The blockage of Cys (β 93) furnishes a satisfactory explanation to the slow-moving electrophoretic migration of Hb Ube-1. This kind of blockade abolishes the electrolytic dissociation of Cys SH which bestows a negative charge, and results in the relative increase in positive charge of β chain and slower anodal electrophoretic migration of the relevant hemoglobin.

In addition, the blockage of Cys (β 93) exerts dual influence upon the β chain: (1) The β chain and the hemoglobin holding it becomes unstable, and (2) the hemoglobin becomes more susceptible to spontaneous oxidation of heme iron. Hb Ube-1 is, therefore, more labile than Hb A, aggregates, undergoes denaturation and finally produces Heinz body in erythrocytes.

3) Thalassemia minor. It has for a long time been believed that thalassemia is not found in Japan. At present there is evidence for the presumption that thalassemia minor is distributed among the Japanese people, although it is rarely encountered. Pedigrees of typical thalassemia minor were discovered in Tokyo[15] and Amami-Oshima island.[11]

4) A family of high fetal hemoglobin was found in Hiroshima.[16]

5) Hb Yukuhashi disease[11] should be mentioned as a condition which may be confused with thalassemia because in this condition numerous target cells appears in the peripheral blood in spite of the absence of enhanced hemolysis.

Hb M disease

There are two varieties of Hb M disease in Japan: One is hereditary nigremia

(Tamura-Takahashi's disease) and the other is a cyanotic case reported from Kurume.

1) Hereditary nigremia[5] is a congenital condition characterized by cyanotic coloration of the visible mucous membrane and the skin without any appreciable ill effect on health. The disease has consecutively been existing for the past 160 years in a village called Shinden situating at a distance of about one hour's trip by train from Morioka, Iwate Prefecture. Recently the disease was confirmed to be distributed also in Hokkaido.[17] There are about 70 patients with hereditary nigremia. Since 1950 an extensive study covering all the branches of medical science has been conducted by Tamura and his associates, who came to the conclusion that the etiology of this condition was related to the production of a hemoglobin possessing abnormal porphyrin nuclei extremely prone to spontaneous oxidation yielding hematin-like complex compounds.[18] The possibility of the abnormality of globin moiety had, however, never been imagined about the hemoglobin of nigremia until 1960, when Shibata and his associates demonstrated a chocolate-colored hemoglobin in the hemolysate of the patient by agar gel electrophoresis and identified it as one of Hb M's.[7] This hemoglobin is now called Hb M_{Iwate}.

The chemistry of Hb M_{Iwate} was studied by Shibata and his associates in Japan and[19-21] by Gerald[22] and Lehmann[23] in the United States and England.

Hb M_{Iwate} is separable from Hb A as oxy-Hb type pigment by agar gel electrophoresis (Figure 3), Amberlite IRC 50 chromatography and carboxymethyl cellulose chromatography. It can also be isolated from Hb A by starch block electrophoresis

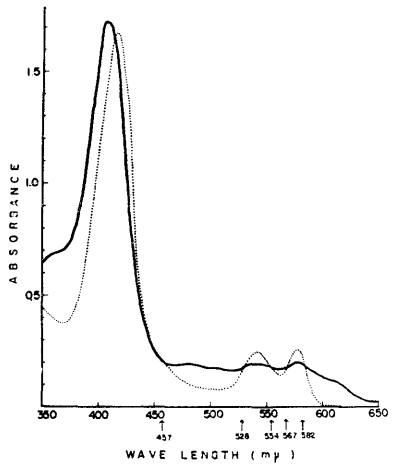

Figure 3. Hb M_{Iwate} (O_2 Hb type) purified by agar gel electrophoresis. Solid line: Hb M_{Iwate}, broken line: Hb A (Hb M_{Iwate} and Hb A are equal in concentration; arrows indicates isobestic points)

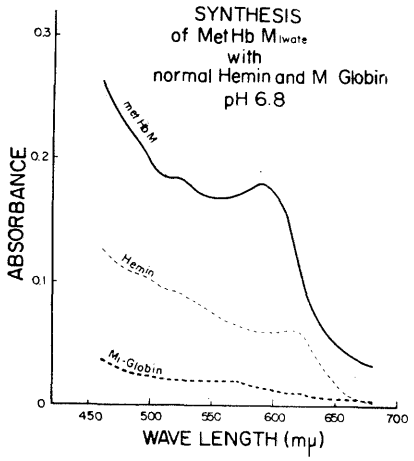

Figure 4. Artificial synthesis of Hb M_{Iwate} from the hemin of Hb A and the globin of Hb M_{Iwate}. The final product is met Hb M_{Iwate}.

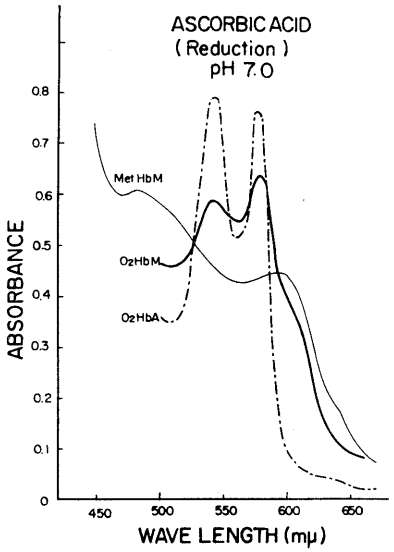

Figure 5. Met Hb M_{Iwate} synthesized from normal hemin and the globin of Hb M_{Iwate} was reduced to Hb M_{Iwate} (O_2 Hb type) with ascorbic acid. O_2 Hb A which was similarly synthesized is presented for the sake of comparison.

after the hemolysate has been treated with ferricyanide to convert oxy-Hb pigment into met Hb pigment.

There is no room for doubt that Hb M_{Iwate} is a hemoglobin composed of a globin of abnormal α chain and normal heme, because two sets of experiments described below clearly demonstrated its validity.

(a) Artificial synthesis of Hb M_{Iwate} from normal hemin and the globin of Hb M_{Iwate}: Hb M_{Iwate} (O_2 Hb Type) was purified by agar gel electrophoresis, and its heme was removed by Anson-Mirsky's technique to obtain its purified globin. The globin was mixed with the hemin prepared from the hemoglobin of a normal person. The mixture was allowed to stand at low temperature for a while and reduced with ascorbic acid to get oxy-Hb type of synthetic pigment (Figures 4 and 5). The absorption curve of the mixture was examined in a Beckman spectrophotometer. The curve was entirely identical with that of Hb M_{Iwate} (O_2 Hb type). It is therefore, presumable from this experiment that Hb M_{Iwate} is a combined product of normal heme and abnormal globin.

(b) Hybridization of Hb M_{Iwate} (O_2 Hb type) with canine hemoglobin ($\alpha_2^{Can}\beta_2^{Can}$): Hb M_{Iwate} and canine hemoglobin (Hb Can) were mixed in equal amounts and subjected to dissociation (acid medium) and recombination (alkaline medium). The hybrid hemoglobins thus formed were examined by starch gel electrophoresis. Four stripes, Black ($\alpha_2^{MI}\beta_2^{Can}$), Red (Hb Can), Black (Hb M_{Iwate}) and Red ($\alpha_2^{Can}\beta_2^{A}$) lining up from the cathode side to the anode side in the order mentioned were seen on the

starch gel plate (Figure 6). The black stripes are concerned with the hemoglobins possessing the α chain of Hb M_{Iwate}, stripes of the hemoglobins having the β chain of Hb M_{Iwate} were red. It is accordingly obvious that the α chain of Hb M_{Iwate} is black in color and abnormal, while its β chain is red and normal.

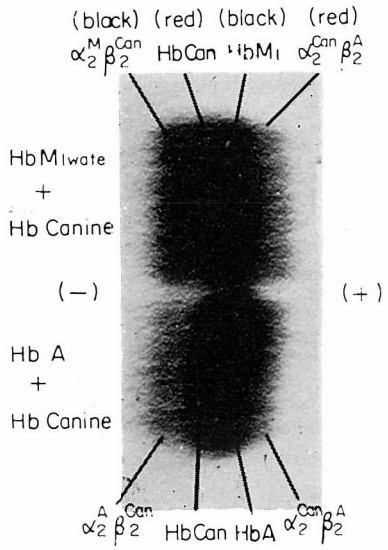

Figure 6. Hybridization of Hb M_{Iwate} (O_2 Hb type) with Hb Can. The hybrids were separated by starch gel electrophoresis.

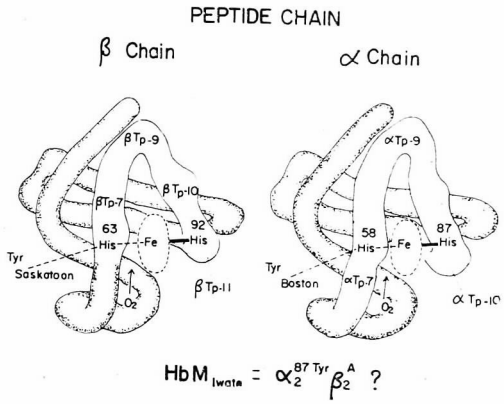

Figure 7. Presumptive amino acid substitution in the α chain of Hb M_{Iwate} as shown by three-dimensional models of α and β chains. Amino acid substitution in the β chain of Hb M_{Kurume} (Hb $M_{Saskatoon}$) is presented for the sake of comparison.

The abnormal spot of the α chain of Hb M_{Iwate} was distinctly visible on the fingerprint and it was extracted for amino acid analysis. The result disclosed almost completaly that in Hb M_{Iwate} the 87th amino acid (histidine) of the α chain was substituted for by tyrosine. Hb M_{Iwate} is therefore $\alpha_2^{87Tyr}\beta_2^A$ (Figure 7).

2) Cyanotic case found in Kurume.[24] This is concerned with a 6 year old boy who was cyanotic since his birth. At the age of 3, he was diagnosed as having cardiovascular abnormality. During his infancy he has never had dyspnea and palpitation. His father and mother were entirely normal. At 6, he was seen by Kimura[24] in the Department of Internal Medicine, Kurume University School of Medicine. The diagnosis of Hb M disease was entertained on the basis of the result of the spectrophotometric examination of the hemolysate (acid met-Hb type).

The abnormal hemoglobin was for the first time demonstrated by starch block electrophoresis of the met-Hb type hemoglobin.[25] This hemoglobin was designated Hb M_{Hida} or Hb M_{Kurume}. Its chemical study was accomplished by Shibata and his associates who confirmed that it was $\alpha_2^A\beta_2^{63Tyr}$, which was identical with Hb $M_{Saskatoon}$.[26] Unlike Hb M_{Iwate}, the O_2 Hb type pigment of Hb M_{Kurume} could not

be differentiated from Hb A by agar gel electrophoresis.

Hb M's in Japan and those in the world

No less than ten varieties of Hb M have been recorded in the literature all over the world for the recent several years. They are classified by the difference in the shape of the absorption curve of the acid met-hemoglobin type hemolysate into three groups, namely Hb M_{Boston} type, Hb $M_{Saskatoon}$ type and Hb $M_{Milwaukee\text{-}1}$ type.[27]

Hb M_{Iwate} belongs to the M_{Boston} type,[28,29] while Hb M_{Kurume} to the Hb $M_{Saskatoon}$ type.[29] Recently a new variety of Hb M named Hb $M_{Kankakee}$ was discovered in Chicago by Heller and his associates.[30] Cross-check experiment of Hb M_{Iwate} and Hb $M_{Kankakee}$ carried out in Chikago and Ube revealed that these hemoglobins are identical.

CONCLUSION

It is extremely interesting from the stand point of anthropology that (1) Hb M has never been detected from the people living in the equatorial and tropical countries among whom hemoglobinopathies are frequent, (2) its distribution is limited to the Caucasian races, particularly of th German ancestry, and the Japanese, and (3) Hb M_{Iwate} and Hb M_{Kurume} are owned in common by the Caucasians and the Japanese.

It will be worthy of mention that Hb E which is widely distributed in the countries of South Eastern Asia has not been detected in Japan.

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