

Thalassemia Mutations and Their Clinical Aspects in Japan

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Abstract

Thalassemia (thal) is one of the most prevalent congenital disorders in a world, especially in an endemic area of *Plasmodium falciparum*. The thal is relatively rare in northeast Asia including Japan where malaria is uncommon. However, thal in Japan has peculiar mutation spectrum and characteristics. Most β -thal patients in Japan are heterozygote and thal minor as a phenotype. They are prone to be misdiagnosed as iron deficiency anemia. Thirty-four mutations of β -thal were thus far identified, and ten of which comprise 80% of β -thal carriers. Among them 60% are unique to Japanese and 40% possibly from abroad. The exception is homozygote for -31G-A which leads to thal intermedia by β^+ -thal phenotype. More than a half of patients with α -thal are of Southeast Asian type, but mutations of the remaining patients seem to be unique to Japanese and yet undetermined. Thus, Japanese thal's have dual origin. The frequency of β -thal is one in 600~1,000 of general population, and that of α^+ -thal ($-\alpha/$) is one in 400. Thus, α -thal trait ($-\alpha/\alpha$) is extremely rare. Another α -thal trait ($--/\alpha\alpha$) would be one fifth of β -thal. Seventeen families of HbH disease ($--/\alpha$) were found. Many of them are related to Southeast Asian. Cases of non-iron deficient microcytosis and positive in the screening for hemoglobinopathies are subjected to gene analysis using allele-specific PCR, SSCP, direct sequencing and gap PCR. Precise breakpoints with large deletion are being identified by gene dosage and PCR instead of conventional Southern blotting and cloning. Most Japanese thal's are asymptomatic (or not hemolytic) except for microcytosis. However, dominant-type β -thal (or Heinz body β -thal) are found, and their clinical phenotype vary with mutations. Some of them become symptomatic transiently. This is also seen in β -thal coexisting with α -triplication. Acute exacerbation or transient appearance of Heinz body is seen in ordinary thal mutations on physical conditions such as pregnancy.

Thalassemia (thal) is one of the most prevalent congenital disorders in a world, especially in an endemic area of *Plasmodium falciparum*. It is relatively rare in northeast Asia including Japan where malaria is uncommon. However, thal in Japan might deserve to mention with its peculiar mutation spectrum and characteristics, and is introduced here.

1. Characteristics of Japanese Thals

1.1. Mutation Spectrum

Most of β -thals occur by point mutation as those found in other countries. The mutation spectrum of thal is as follows: -31 A-G (16.3% of all β -thal carriers),

codon 90 GAG-TAG (15.0), IVS-II-654 C-T (13.7), IVS-I-1 G-A (12.7), codons 41/42 TTCTTT-TT (8.3%), codons 127/128 CAGGCT-CCT (4.4), initiation codon ATG-GTG (2.8), codons 84/85/86 TTT-TTTT (2.8), codon 121 GAA-TAA (2.6), codon 17 AAG-TAG (2.3), and other 24 rare mutations (19.1). The top 10 mutations account for 80% of all patients with β -thal. This finding facilitated the gene diagnosis performed in our laboratory at present. Most of α -thals are caused by large gene deletion including α -globin gene. Among them Southeast Asian-type ($--$ -SEA) comprises more than half of the patients (58%), and followed by Filipino-type ($--$ -FIL) (5%). Remaining 37% seem to have a variable size of a large deletion whose precise breakpoints still remain unresolved. They are sporadic

and possibly unique to Japanese.

1.2. Origin

The most common mutations of Japanese β -thal, 31 A-G and codon 90 nonsense, are unique to Japanese, and not found in other ethnic groups. Other mutations such as initiation codon ATG-GTG, -3 codons 127/128 and +1 codons 84/85/86 are also exclusively found in Japanese. These mutations comprise about 60% of all β -thal individuals. These mutations would have appeared as being neutral for gene selection. The other 40% would be brought in from endemic areas overseas. The 4 codons 41/42 and IVS-II-654 C-T are examples of them. The α -thal is also like this, and SEA and FIL would be carried from abroad. Thus, Japanese β -thal mutations seem to be dual origin. Japan is an island, and immigrant from southern part of China and Southeast Asia where β -thal is endemic, has presumably come ashore historically.

1.3. Frequency

The frequency of β -thal varies in districts, but one in 670-1,000 for general population is reported. The twenty-one families homozygous for 31 A-G in a TATA box are discovered mainly in Tohoku district, northeast Japan. Since this homozygosity is not clinically severe (β^+ -thal), they survived and would have given birth to their descendents. This would be the cause that -31 A-G is commonest among Japanese β -thal carriers. Frequency of α^+ -thal chromosome (- α) seems to be one in 400 individuals. Thus, α -thal trait (- α / α) are extremely rare. However, another α -thal trait (--/ $\alpha\alpha$) whose exact frequency is not yet known, seems to be one fifth of β -thal by the number referred to our laboratory. HbH disease (--/ α) is discovered in 17 families, most of which are related to Southeast Asian. Recently α -thal in Japan is often found in Southeast Asians staying in Japan.

1.4. Divergence of Thal Chromosomes (haplotypes)

The -31 A-G revealed two kinds of haplotypes that linked to $\Lambda_{\text{V}}^{\text{T}}$ gene. A regression analysis against historical increase in the Japanese population suggests that it occurred more than 2000 years ago. Haplotypic divergence might support such a long history. In contrast, codon 90 (nonsense) reveals no divergence of haplotype, suggesting relatively recent event of occurrence. Several haplotypes are found in -4 codon 41/42 that are prevalent among Asian.

2. Methods

2.1. Screening

Since most microcytosis without iron deficiency has high possibility of β -thal in Japan, exclusion of iron deficiency anemia is prerequisite for proceeding to screen-

ing examination for β -thal. The conventional screening programs composed of quantification of HbA2 and HbF, searching for inclusion bodies, resolution of abnormal band by isoelectric focusing and GLT1/2, are effective to suspect thals as well as their discrimination. As Japanese β -thal mutations are relatively heterogenous, this step is essential for gene analysis. Some of them, however, remain genetically undetermined.

2.2. Gene Diagnosis

The phenotype, residence and ethnic group of the patient are important information that suggests the possible mutation. They are surveyed by allele-specific PCR including 10 common mutations in case of Japanese. Otherwise, SSCP is performed and followed by direct sequencing. When α -thal is suspected, --SEA, --FIL and - $\alpha^{5,7}$ are surveyed by gap PCR.

2.3. New Approach to Deletion Types

A number of deletion type of β -thal, especially of α -thal, remain without identification of the breakpoints. Southern blot analysis has been one of conventional tools for these cases. However, recent technological innovation enabled rather accurate and simple estimation of deleted DNA segment. Using LightCycler (Roche Diagnostics Inc, Japan), rough estimation of deletion is achieved. Subsequently, recombination of genomic DNA and M13 vector both being digested by eight kinds of endonucleases, is followed by PCR with the M13 primer and appropriate primers near the breakpoint. Abnormal fragments are looked for in electrophoresis by comparing with those of normal control. Sequencing of them unravels the precise breakpoints yet unknown. A new Japanese $\delta\beta$ -thal was identified in this way in five unrelated Japanese families.

3. Clinical Aspects

1) Most β -thal in Japanese is heterozygous and β -thal minor as a phenotype that shows no overt hemolysis but slight anemia with marked microcytosis. Thus, β -thal is recessive in respect to hemolysis, but might be dominant in morphology. The exceptions in Japanese are homozygote for β^+ -thal (-31 G-A), and HbH disease. They are β -thal intermedia with moderate anemia.

2) Some dominant-type β -thal's that reveal hemolytic involvement as a dominant trait, are found. They are also called Heinz body thalassemia, for Heinz bodies are often detected. The typical dominant-type β -thal in Japan is seen in three frameshift mutants in the 3rd exon, ie, -A codon115, Hb Makabe (-A codon123) and Hb Hradc Kralove (-A codon115). However, they are extremely rare. Some cases of Hb Gunma (-3 codons 127/128) and codon 121 GAA-TAA (nonsense) have Heinz bodies with evidence of hemolysis, while others of them show β -thal minor. Mutation at codon 131 have moderate anemia, but doesn't have Heinz bodies. Hb Showa-Yakushiji (codon 110 T-C), a superunstable

variant, is categorized as dominant type but demonstrates a phenotype of β -thal minor. No Heinz body is observed. However, they tend to have relatively lower Hb levels than usual β -thal minor. Thus, the clinical severity varies by mutation. Of particular interest are findings common to all these dominant-type thals in Japanese that moderate to severe anisopoikilocytosis is present. This picture is also seen in transient exacerbation described below.

3) Particularly stressed are mutants such as -4 codons 41/42, codon 90 nonsense, codon 15 nonsense, IVS-II-1 G-A which are expected to give a typical β -thal minor. However, some of them occasionally do have Heinz body accompanied by marked anisopoikilocytosis. This acute exacerbation might be induced by acquired

factors, such as pregnancy and infection. The reason of it is obscure.

4) Presence of a triplicated α -globin gene coexisting with β -thal mutation might exacerbate the phenotype of β -thal minor by exaggeration of the imbalance of β/α globin synthesis. Nevertheless, this phenomenon is still in a controversy, for a number of such cases reveal a typical β -thal minor. The β -thal individuals with triplicated α -globin gene as well as Hb Gunma and codon 121 nonsense described above, usually show β -thal minor but might be vulnerable to alteration of physical conditions, which brings about transient exacerbation characterized by appearance of Heinz bodies.