MINISTRY OF EDUCATION AND TRAINING
MINISTRY OF HEALTH
HAI PHONG UNIVERSITY OF MEDICINE AND PHARMACY

DO THI QUYNH MAI

DETECTION OF SOME GENE MUTATIONS AND GENOTYPE-PHENOTYPE CORRELATION OF THALASSEMIA PEDIATRIC PATIENTS AT HAI PHONG CHILDREN'S HOSPITAL

Specialization: Pediatrics
Code: 97.20.106

SUMMARY OF DOCTORAL THESIS

HAI PHONG- 2022

THE WORK WAS COMPLETED
AT HAI PHONG UNIVERSITY OF MEDICINE AND PHARMACY

SCIENTIFIC INSTRUCTORS

1. Assoc.Prof.Dr. Nguyen Ngoc Sang

2. Dr. Bach Thi Nhu Quynh

Reviewer 1: Assoc.Prof.Dr Nguyen Tien Dung

Reviewer 2: Assoc. Prof.Dr Nguyen Ha Thanh

Reviewer 3: Assoc. Prof.Dr Vu Thi Thuy

The thesis was defended at the School-level Thesis Judging Committee at Hai Phong University of Medicine and Pharmacy.

At ……hour ……min, date 12 year 2022

The thesis can be found at:

National Library

Library of Hai Phong University of Medicine and Pharmacy

POSED ISSUES

Thalassemia is a group of congenital hemolytic anemia caused by mutations in the globin gene that cause partial or complete deficiency in polypeptide synthesis. Currently, about 7% of the world's population carries the Thalassemia gene. The disease affects most countries, but there are significant differences even in small geographical areas. Vietnam is located in the "Thalassaemia belt" According to statistics, our country is one of the countries with the highest rate of Thalassemia patients in the world. In Hai Phong, the problem of diagnosis and management of Thalassemia has been carried out but has not really been able to control and control the disease. From there, it leads to the transmission of disease-causing genes from generation to generation, degrading the species and bringing many serious consequences to the family and society.

From the above facts, we always wonder: What is the gene mutation of Thalassemia pediatric patients in Hai Phong? How effective has the management of this disease genetic resource been? This is very necessary and very important, contributing to creating the foundation for proactive prevention, taking disease prevention solutions as a strategy to solve Thalassemia problems, then can initially eliminate and reduce the genetic resources that cause Thalassemia. So we made the theme : "***Detection of some gene mutations and genotype-phenotype correlation of thalassemia pediatric patients at hai phong children's hospital"*** with the following three goals**:**

1, Identifying some genetic mutations of Thalassemia pediatric patients at Hai Phong Children's Hospital from January 1, 2016 to December 31, 2020.

2, Defining genotype-phenotype correlation of Thalassemia pediatric patients at Hai Phong children's hospital.

3, Describing the clinical and subclinical characteristics according to the mutant gene and initially building a number of pedigrees of Thalassemia children at Hai Phong Children's Hospital

NEW CONTRIBUTIONS OF THE THESIS

The study determined the rate of disease-causing gene mutations and genetic characteristics of the disease through studying some genealogies of Thalassemia patients being treated at Hai Phong children's hospital. Giving the combination of molecular biology techniques to determine the type of mutation and other tests (total blood cell analysis, blood biochemistry, ...) to evaluate and contribute to the screening of Thalassemia disease. Detecting rare mutations in the world in general and Vietnam in particular

The research is quite comprehensive on Thalassemia disease: Disease causes (mutant types), inheritance through generations, clinical and subclinical symptoms, treatment and monitoring of treatment results

STRUCTURE OF THE THESIS

The main part of the thesis is 139 pages long, including the following parts: Introduction: 2 pages; Chapter 1- Overview: 36 pages; Chapter 2 - Research subjects and methods: 16 pages; Chapter 3 - Research results: 39 pages; Chapter 4 - Discussion: 37 pages; Conclusion: 2 pages; New contributions: 1 page; Limits: 1 page; Recommendation: 1 page; Further research direction: 1 page. The thesis has 148 references, of which 60 are in Vietnamese and 88 in English. The thesis has 29 tables, 24 figures, 2 diagrams.

Chapter 1. OVERVIEW

1.1. Epidemiological features of Thalassemia disease

1.1.1. Epidemiology

Thalassemia is one of the most common genetic disorders in the world, the disease is related to ethnic origin. The disease is globally distributed, but geographical distribution, and is common in the Mediterranean region, the Middle East, Southeast Asia, and North Africa. According to a report by the International Thalassemia Federation, the number of people carrying the Thalassemia gene makes up about 7% of the global population. In Southeast Asia in general and Vietnam in particular, the rate of people carrying the Thalassemia gene is high.

Currently, the phenomenon of interference between ethnic groups and geographical displacement causes disease genes to spread and increase significantly throughout the country. In big cities, many people carry disease genes.

1.1.2. Mechanism of inheritance of Thalassemia

α-Thalassaemia disease is caused by a mutation in the gene synthetically encoding the α-globin chain, resulting in a decrease or absence of the α-globin chain in the hemoglobin molecule. The decline in synthesis leads to an excessive increase in the synthesis of the β-globin chain forming molecules γ4-called Hb Bart's (during fetal life) and β4- called HbH (during adulthood). The α-globin chain is synthesized from 4 genes, including 2 HBA1 genes and 2 HBA2 genes. The number of α-globin chains depends on the number of active genes. The α-thalassemia gene region is located on the short arm of chromosome 16 (16p13.3), each chromosome has 1 HBA1 gene (alpha 1) and 1 HBA2 gene (alpha 2). The HBA1 gene is 840bp in length and consists of 3 exons and 2 introns. The HBA2 gene is 830bp in length and consists of 3 exons and 2 introns. The large segment loss in the form of SEA accounts for over 90%.

α-Thalassaemia disease is inherited by autosomal recessive allele rule on autosomes. Genetic mechanism: the disease can be caused by disease genes passed from parents to children or by new mutations arising through the process of creating gametes in parents into the offspring. The child will receive one 16^{th} chromosome carrying 2 HBA genes from the mother and one 16^{th} chromosome

carrying 2 HBA genes from the father, so the risk of alpha Thalassemia in the child depends on the number of mutated genes that the child received from the parents.

β-Thalassaemia disease occurs due to point mutations in locus producing β-chain that reduce or lose function of genes coding for β-globin synthesis, resulting in decreasing or not having synthesis of β-globin chains. The HBB gene region that regulates beta globin synthesis is located on the short arm of 11th chromosome (11p15.5), 1600bp long, consisting of 3 exons and 2 introns. When there is a mutation in the HBB gene, there is a decrease or no production of the β-globin chain of hemoglobin. Today, more than 300 mutations have been found in the HBB gene, including 2 groups: the group that completely loses the function of the HBB gene leading to the failure to produce the β globin chain and the group that reduces the production of the β globin chain.

β-Thalassaemia disease is inherited by autosomal recessive allele rule on autosomes. Genetic mechanism: The disease can be caused by disease genes passed from parents to children or by new mutations arising through the process of creating gametes in parents into the offspring. The HBB gene locus is located on 11th chromosome. The child will receive one 11th chromosome carrying 1 HBB from the mother and one 11th chromosome carrying 1 HBB from the father, so the risk of β-Thalassaemia in the child depends on the number of mutated genes received from parents.

1.2. Clinical features and laboratory

Thalassemia classification: Depending on deficiency of α, β chain synthesis or deficiency of both β and δ chain, it is classified as α-Thalassaemia, β-Thalassaemia, δβ-Thalassaemia.

1.2.1. α-Thalassaemia

Depending on the different combinations between the two mutant alleles α0 and α+-thalassemia, different phenotypes of α-thalassemia are produced.

α-thalassemia disease is divided into 4 types according to the number of α globin genes that are mutated, with various clinical manifestations.

The form of α+-Thalassaemia is a person who loses a gene on the chromosome (-α/αα), without clinical symptoms. The analysis of the α globin gene is carried for definitive diagnosis.

There are two types of α0-Thalassaemia: The Cis form is a deletion of two segments of one gene on one chromosome (--/αα).

The Trans form is a deletion of two segments of one gene on two homologous chromosomes (-α/-α). Mild manifestations, often without clinical symptoms, are detected only through blood tests.

- HbH disease is a symptomatic α-Thalassaemia form (double heterozygous α0-α+-thal) consisting of a mutation with a deletion of two genes

and a mutation with a deletion of one gene. Clinical symptoms of anemia, HbH 0.8-40% (sometimes Hb Bart's), splenomegaly, jaundice depending on the degree, growth retardation, manifestations of iron excess.

- Hb Bart's hydrops fetalis syndrome is the most severe form of the disease, losing all 4 α globin genes, causing a complete decline in the ability to produce α globin chains. Hb Bart's pregnancy is edema, heart failure, prolonged anemia, hepatosplenomegaly, brain retardation, abnormal bone and cardiovascular system development, thick placenta. Most Hb Bart's will die during pregnancy or postpartum.

1.2.2. β-Thalassaemia disease

- The major β-thalassemia with homozygous genotype, clinical symptoms of severe chronic hemolytic anemia (anemia, jaundice, splenomegaly, deformity bone, physical retardation, iron infection...). The red blood cell index shows severe anemia, hypochromic small red blood cells, severely deformed with many irregular large and small red blood cells, many ring-shaped, target-shaped, teardrop-shaped red blood cells… having the changes in Hb composition on solid electrophoresis signs (HbF increases, HbA1 severely reduced/no longer present, HbA2 increases).

- Intermedia β-Thalassaemia form inherits two slight β + mutations, often has a combination with α-Thalassaemia. Clinically, there is moderate or mild anemia, splenomegaly, jaundice, manifested bone deformity and little physical retardation, late manifestation of iron infection. Laboratory test shows moderate anemia. Red blood cells change as in serverse form.

Hb components on electrophoresis shows that HbF increases from 20-100%, HbA1 accounts for 0-80%, HbA2 is about 7%.

- The mild β-Thalassaemia form is heterozygous β-Thalassaemia, the genetype can be β + / β or β0/β. This form of disease, the patient's body develops normally, there is no bone deformity, anemia is usually mild.

The Hb components on electrophoresis are commonly seen that HbF increases from 1-10%, HbA1 accounts for > 80%, HbA2 is about 3.5% - 8%.

1.2.3. Overview of methods to detect genetic mutations that cause Thalassemia

- The RE-PCR method bases on the principle that when a mutation is generated, it can form or destroy the cutting position of the restriction cutting enzyme. In the experiment, the product after amplification by PCR reaction is cut by restriction cutting enzyme. Depending on the calculation, the enzyme will cut or not cut the DNA strand when the mutation point is presented.

- MLPA is a technique for quantifying changes of the number of copies of chromosomal DNA. This method bases on multiplex-PCR reaction in combination with pre-designed probes for target genes to detect deletions as well as changes in the number of copies of genes.

- DNA hybridization is a technique combining multiplex-PCR with DNA hybridization to detect many different mutations at the same time.

- Realtime PCR is a technique that uses fluorescence to quantify the number of copies of the PCR product through each cycle. When compared with standards of known concentration, basing on the correlation between the time and the intensity of the fluorescence emitted, the number of copies of the target sequence in the original sample can be inferred.

- DNA sequencing is a method of reading the entire sequence of the gene segment of interest, from which any changes occurring in the gene segment can be identified. Depending on different analytical systems, each read segment can be from 50-600 nucleotides long (2-NextSeq generation machine) or 800-900 nucleotides (1-generation machine bases on Sanger principle).

- Gap-PCR allows identifying deletion mutations or rearrangements on DNA molecules by using at least 3 primers (2 forward and 1 reverse primers), of which the first primer pair is located at the two ends of the missing DNA segment, the remaining primer is designed to supplement the missing region of segments.

- ARMS bases on the principle of PCR reaction using allele-specific primers. The primers used in the reaction are designed so that the 3' end of the primer is paired at the position where a point mutation can occur.

- Multiplex-PCR is a modified PCR technique, involving multiple primer pairs in a reaction to amplify multiple target gene sequences. Each reaction uses a specific primer with a 3'-terminal sequence added to the mutant allele and a common primer opposite to the allele-specific primer. The presence of mutant alleles is represented by amplified DNA product of different sizes which are known.

Chapter 2. RESEARCH SUBJECTS AND METHODS

2.1. Subjects, time, and place of the study

The study subjects includes 85 patients with a confirmed diagnosis of Thalassemia (including 27 α-Thalassaemia patients and 56 β-Thalassaemia patients and 2 patients carried alpha and beta genes) during the study period from January 1, 2016 to December 31, 2020 at the Department of Nephrology, Blood and Endocrinology, Hai Phong Children's Hospital.

2.1.1. Criteria for patient selection

- Diagnosis of Thalassemia:

Clinical: Full symptoms of chronic hemolytic anemia (anemia, jaundice, hepatosplenomegaly, bone deformities...), physical retardation and iron infection (hepatomegaly, liver failure, enlarged heart, heart failure…)

Laboratory tests: Changes in specific Hb composition to each disease: Hb electrophoresis is abnormal.

+ Severe β-thal: HbF increases, HbA1 decreases, HbA2 is normal or increasing.

• β0-thal: HbA1 is zero, HbF is very high > 90%, HbA2 > 4%

• β+-thal: HbF, HbA2 > 4%, HbA1 severely reduces.
+ HbE/β-thal disease: HbF increases by 10%, HbE >10%, HbA1 decreases,
• HbE/β0-thal: HbA1 is zero, Hb electrophoresis shows only HbF and HbE
• HbE/β+-thal: HbF increases, HbA1 decreases, HbE >10%,
+ HbH disease has HbH, HbA1 usually decreases. HbA2 may be reducing or normal.

2.1.2. Exclusion criteria

- The patient's family does not consent to participate in the study

2.2. Research Methods

2.2.1. Study design: A prospective, descriptive study of cases series

2.2.2. Study sample size: All 83 patients with confirmed diagnosis of Thalassemia are being treated as inpatients and outpatients at the Department of Nephrology - Blood - Endocrinology at Hai Phong Children's Hospital and the patient's family during the study period, being consistent with the inclusion and exclusion criteria of the disease group presented above. The method of choosing a utility template.

2.2.3. Research indicators and variables

2.2.3.1. Objective 1: **Identifying gene mutations causing Thalassemia in pediatric patients at Hai Phong Children's Hospital**

Age of hospitalization, age of onset of disease, duration of disease up to the time of study, sex, geographical location (urban/rural), identification of genetic mutations causing Thalassemia.

2.2.3.2. Objective 2: **Defining genotype-phenotype correlation of Thalassemia pediatric patients at Hai Phong children's hospital**

The reason for hospitalization, clinical symptoms (anemia, jaundice, manifestations of iron infection, hepatosplenomegaly, bone deformities, Thalassemia face), the number of blood transfusions/year, transfusion dependence. Testing for the total analysis of peripheral blood cells, quantifying ferritin, serum iron, GOT, GPT, urea, creatinine.

2.3. News data collection tools and processing results

SPSS software version 26.0 (SPSS Inc., Chicago, Illinois) is used to process the statistical results.

2.4. Ethics in research

The research theme strictly adheres to research ethics in Medicine. The theme has been approved by the Facility Protection Council of Hai Phong University of Medicine and Pharmacy, the Medical Ethics Council of Hai Phong Children's Hospital. The theme has the consent of the parents and guardians of the study subjects, they were explained, advised and committed to voluntarily

participate in the study in documents, the patient's information are guaranteed to be kept confidential.

Chapter 3. RESEARCH RESULTS

Out of 85 patients, 2 patients carried both gene mutations of alpha and beta thalassemia. Therefore, when studying clinical symptoms, the research only performed on 83 patients

3.1. Genetic mutations cause thalassemia in pediatric patients at Hai Phong Children's Hospital

3.1.1. General characteristics of the study subjects

- The average age of hospitalization is 6.93 ± 5.00 years old,
- The average age at diagnosis is 1.86 ± 2.52 years old.
- The male/female ratio of the disease is 43/40 (1,075: 1).
- Mainly Thalassemia patients live in rural areas p>0.05.

Table 3.1. Distribution of thalassemia pediatric patients by age group at hospital admission

Age group	α-Thalassemia n(%)	β-Thalassemia n(%)	In common n(%)	p (*test* χ^2)
0-<1	5(18,5)	5(8,9)	10(12,0)	
1-<5	7(25,9)	11(19,6)	18(21,7)	
5-<10	8(29,6)	18(32,1)	26(31,3)	0,611*
10-<15	6(22,2)	19(33,9)	25(30,1)	
≥15	1(3,7)	3(5,4)	4(4,8)	
Total	27(31,8)	56(65,9)	83(100,0)	

Comments: Most of the patients admitted to the hospitalization were between the ages of 1 and 15 years old. Children with α-Thalassaemia admitted to the hospitalization were found quite evenly in all ages, while children with β-Thalassaemia were mainly found in the 5-<15-year-old age group. There was no difference in age of admission between the study groups, p>0.05.

Table 3.2. Distribution of patients according to age at diagnosis

Age group	α-Thalassemia n(%)	β-Thalassemia n(%)	In common n(%)
<1 y	8(29,6)	24(42,9)	32(38,6)
1-<5 y	15(55,6)	24(42,9)	39(47,0)
5-<10 y	4(14,8)	5(8,9)	9(10,8)
≥10 y	0(0,0)	3(5,4)	3(3,6)

Total	27(32,5)	56(67,5)	83(100,0)

Comments: Most Thalassemia patients were diagnosed at the age of <5 years (accounting for 85.6%). 42.9% of β-thalassemia patients were diagnosed <1 year old, 42.9% of β-thalassemia patients were diagnosed from 1 to <5 years old. Meanwhile, 51.9% of α-Thalassaemia patients detected the disease from 1 to <5yrs old and 29.6% of α-Thalassaemia children detected the disease < 1 year old.

3.1.2. Identifying genetic mutations causing Thalassemia in pediatric patients at Hai Phong Children's Hospital

- There were 67.5% children with β-Thalassaemia and 32.5% of children with α-Thalassaemia.

Table 3.3 Distribution of hemoglobin gene mutations in α Thalassaemia children

Gene mutation	Number of patients	Ratio (%)
3.7	1	3,7
3.7 – SEA	3	11,2
C2 deIT	1	3,7
HbCs	2	7,4
HbCs – SEA	9	33,3
SEA	9	33,3
SEA – C2 delT	2	7,4
Total	27	100,0

Comments: Among the studied pediatric patients, α-Thalassaemia patients carried mutations in the hemoglobin genes of HbCs - SEA and SEA, accounting for 66.6%. Other gene mutations accounted for a small percentage.

Table 3.4. Classification of α-Thalassaemia pediatric patients by BG type (n = 27)

Mutant type	Mutant Gene	Number of mutant alleles	Ratio (%)
The deletion of segment	SEA	23	56,1
	3.7	4	9,8
Non- deletion of segment	HbCs	11	26,8
	C2 deIT	3	7,3

Comments: We found 41 mutant α-Thalassaemia alleles, SEA mutation accounted for the highest rate 56.1%, followed by HbCs accounted for 26.8%.

- There were 85.2% of α-Thalassaemia patients with more than 1 gene mutation.

Table 3.5. Distribution of β-globin gene mutations in β-Thalassaemia patients

Mutation of the β-globin gene.	Phenotype	Number of mutant alleles	Ratio (%)
CD 41/42 (- TCTT)	β^0	21	25,3
CD 17 (AAG – TAG)	β^0	15	18,1
CD 26 (GAG – AAG)	β^E	33	39,8
CD 71/72 (+ A)	β^0	13	15,7
CD 95 (TAC – TAA)	β^0	3	3,6
IVS I-1 (G – T)	β^0	1	1,2
Total		86	100,0

Comments: The study detected 86 mutant alleles in the Hb gene of 56 β-Thalassaemia patients. Among them, there were patients with a combination of 2 mutations. The mutation detection rate was 100% of patients entering treatment. The study detected 6 types of β-Thalassaemia mutations, namely CD26 (GAG – AAG), CD41/42(-TCTT), CD17(AA–TAG) and CD71/72(+A) with the corresponding rate of 39 .8%, 25.3%, 18.1% and 15.7%, CD 95(TAC - TAA) accounted for 3.6% and IVS I-1 (G - T) with 1.2%.

Table 3.6. Classification of β-globin mutations according to the location of the mutated gene

Mutant position	**Number of patients**	**Ratio (%)**
Exon 1 (CD17, CD26)	18	32,1
Exon 2 (CD41/42,CD 71/72, CD 95)	13	23,2
Exon 1 + Exon 2	24	42,9
Intron 1 (IVS I-1) + Exon 1	1	1,8
Promoter region	0	0,0
Tổng (Total)	**56**	**100,0**

Comments: The detected mutations occurred in many gene positions, the most common in 2 exons (exon 1 + exon 2) accounted for 42.9%, exon 1 accounted for 32.1%, exon 2 accounted for 23.2% , intron 1 + exon 1 accounted for 1.8%. There were no cases of mutations in the promoter region of the gene.

Table 3.7. Distribution of β-Thalassaemia children according to mutated gene function

Gene function	Number of patients	Ratio (%)
Transcriptional mutants Promoter regulatory elements - 28 (A – G)	0	0,0

- 88 (C – T)		
Mutations in RNA complete processing Connector position IVS 1 – 1 (G – T) IVS 1 – 5 (G – C) IVS 2 – 654 (C – T)	1 1	1,2 1,2
RNA translation mutation Nonsense codon CD17 (AAG – TAG) CD26 (GAG – AAG) CD95 (TAC – TAA) - Frameshift CD41/42 (-TTCT) CD71/72 (+A)	84 15 33 2 21 13	97,6 17,4 38,3 2,4 24,4 15,1
Other less common mutations (mutants lose exon 2 through the 95 cd position)	1	1,2
Total	86	100,0

Comments: Most of the mutations were related to the RNA translation stage (95.4%). There was 1 mutation in the RNA completion stage (1.2%) and 1 rare mutation (1.2%).

Table 3.8. Distribution of children with β-Thalassaemia by mutant genotype (n = 56)

Genotype	Mutant combination genotype	Number of pts	Ratio (%)
$\beta^0\beta^0$		9	16,1

	- Homozygous type	5	8,9
	CD41/42 – CD41/42	2	3,6
	CD71/72 – CD71/72	3	5,3
	-Double heterozygote of 2 mutations	4	7,2
	CD41/42 – CD17	1	1,8
	CD17 – CD71/72	1	1,8
	CD17 – IVS I-1	1	1,8
	CD17 – CD95	1	1,8
$\beta^0\beta^E$	-Combination Heterozygous HbE	26	46,3
	CD17 – CD26	5	8,9
	CD41/42 – CD26	12	14,2
	CD71/72 – CD26	8	13,8
	CD95 – CD26	1	1,8
$\beta^0\beta$	- Single heterozygous	21	37,6
	CD17	6	10,7
	CD26	7	12,6
	CD41/42	6	10,7
	CD71/72	1	1,8
	CD95	1	1,8

Comments: Our study detected 15 genotypes with mutations in 56 β-Thalassaemia patients in the study.

- β0β0 genotype had 9 patients, accounting for 16.1%. In which, there were 5 patients with homozygous gene with 2 combination types of mutations: CD41/42 - CD41/42, CD71/72 - CD71/72 and 4 patients with double heterozygote of 2 mutations with 4 combination types of mutations CD41/42-CD17, CD17-CD71/72, CD17-CD95 and CD17-IVS I-1.
- β0βE genotype had 26 patients, accounting for 46.3% with 4 genotypes including CD17-CD26, CD41/42-CD26, CD71/72-CD26, CD95-CD26.
- β0β single heterozygous genotypes had 21 patients, accounting for 37.6% with 5 genotypes CD17, CD26, CD41/42, CD71/72, CD95.

3.2. Genotypic and phenotype comparison of Thalassemia pediatric patients at Hai Phong Children's Hospital

Clinical and subclinical manifestations according to mutant phenotype

- Anemia is the main reason why Thalassemia children are hospitalized (48.1%). There was a statistically significant difference in the reason for hospitalization between the two groups of children α-Thalassaemia and β-Thalassaemia, p<0.01.
- Most children with α-Thalassaemia had grade 1 and grade 2 splenomegaly. β-Thalassemia children had splenomegaly from grade 1 to grade 4 quite similarly, up to 26.2% of β-Thalassemia children had splenectomy.
- Thalassemia face mainly appeared in the form of β-Thalassaemia with 35 children (62.5%). 70.4% of children with α-Thalassaemia didn't have typical Thalassemia facial expression. This difference was statistically significant (p<0.05).
- There was a statistically significant difference with p < 0.05 in the expression of dull gray gingival mucosa between the β-Thalassaemia and α-Thalassaemia groups.

Table 3.9. Distribution of study subjects according to clinical symptoms upon hospitalization admission

Triệu chứng Symptoms	**α-Thalassemia n(%)**	**β-Thalassemia n(%)**	**In common (%)**	**p** (*test* χ^2)
Anemia	11(40,7)	40(71,4)	51(61,4)	0,007
Jaundice	6(22,2)	32(57,1)	38(45,8)	0,003
Skin darkening	5(18,5)	32(57,1)	37(44,6)	0,001
Splenomegaly	15(55,6)	31(55,4)	46(55,4)	>0,05
Big liver	16(59,3)	39(69,6)	55(66,3)	>0,05
Bone deformity	6(22,2)	32(57,1)	38(45,8)	0,003

Comments: The results of our study showed that there were statistically significant differences according to clinical symptoms of anemia, jaundice, skin darkening, and bone deformity between α-Thalassaemia and β-groups of patients. Thalassemia, p < 0.05.

Table 3.10. Distribution of patients by age at initiation of blood transfusion

Age blood transfusion	α-Thalassemia n(%)	β-Thalassemia n(%)	In common (%)	p (*test* χ^2)
<1 yearold	3(23,1)	14(37,7)	17(34,0)	>0,05
1-<3years old	1(7,6)	10(27,0)	11(22,0)	
3-<5years old	6(46,2)	7(18,9)	13(26,0)	
>5 years old	3(23,1)	6(16,2)	9(18,0)	
Total	13(26,0)	37(74,0)	50(100,0)	

Comments: 64.7% of β-thalassemia patients started to need blood transfusion at the age of <3 years, while 69.3% of α-thalassemia patients started to need blood

transfusion at the age of >3 years old. However, this difference was not statistically significant (p > 0.05).

Table 3.11. Characteristics of blood transfusion dependence of Thalassemia children

Blood transfusion characteristics	α-Thalassemia n(%)	β-Thalassemia n(%)	In common (%)	p (*test* χ^2)
Dependence	5(18,5)	34(60,7)	39(47,0)	<0,05
Non- dependence	22(81,5)	22(39,3)	44(53,0)	
Total	**27(32,5)**	**56(67,5)**	**83(100,0)**	

Comments: 47% of patients in the study group depended on blood transfusion; 60.7% of β-thalassemia patients were transfusion dependent, but 81.5% of α-thalassemia patients were not transfusion dependent. There was a difference in blood transfusion characteristics between the 2 groups (p <0.05).

Table 3.12. Test characteristics of study subjects (n=83)

Hematologic-al	α-Thalassemia $\bar{X} \pm SD$ (min-max)	β-Thalassemia $\bar{X} \pm SD$ (min-max)	In common $\bar{X} \pm SD$ (min-max)	p
HC (T/L)	4,6 ± 0,9 (2,7 - 6,5)	3,9 ± 1,2 (1,8 - 6,1)	4,1 ± 1,1 (1,8 - 6,5)	<0,05[b]
Hb (g/dL)	90,5 ± 11,2 (68.4 – 123.0)	86,2 ± 19,1 (44.0 – 127)	87,6 ± 16,9 (44.0 – 127.0)	>0,05[b]
Hct (%)	29,6 ± 3,4 (23,5 - 38,3)	26,9 ± 6,5 (10,0 – 38,5)	27,7 ± 5,8 (10,0 - 38,5)	<0,05[b]
MCV (fL)	65,3 ± 8,9 (48,9 - 85,5)	70,0 ± 8,8 (55,0 - 87,0)	68,5 ± 9,0 (48,9 – 87,0)	<0,05[b]
MCH(pg)	20,2 ± 3,3 (14,9 - 29,5)	23,2 ± 4,9 (17,2 - 49,4)	22,4 ± 4,9 (14,9 – 49,4)	<0,05[b]
Ferritin* (ng/ml)	213 (100,0–315,5)	1067,5 (83,3-2000)	300 (92,0-2000,0)	<0,05[a]
Fe serum (µmol/L)	14,2 ± 7,1 (2,1 – 28,9)	22,9 ± 13,4 (4,8 – 78,3)	20,0 ± 12,4 (2,1 – 78,3)	<0,05[b]
GOT(U/l)*	28,0 (24,0 – 49,0)	36,5 (27,0 – 68,2)	35,0 (25,0-53,0)	<0,05[a]
GPT(U/l)*	21,0 (16,0 – 29,0)	30,0 (18,0 – 70,2)	27,0 (17,0 – 53,0)	<0,05[a]

* *Median (25^{th} – 75^{th}); [a]: Mann – Whitney U test; [b]: Test T*

Comments: The red blood cell count and hematocrit decreased more severely in β-Thalassaemia. MCV and MCH were lower in α-Thalassaemia. The concentrations of GOT, GPT, ferritin and serum iron in the β-Thalassaemia group were higher than those in the α-Thalassemia group. The difference was statistically significant with p<0.05

3.3. Clinical and subclinical manifestations according to mutant genotype and initially building a number of pedigrees of Thalassemia children at Hai Phong Children's Hospital.

3.3.1. Clinical and subclinical manifestations according to mutant genotype

- Most patients with α-Thalassaemia rarely showed symptoms of chronic hemolytic syndrome, hepatomegaly, bone deformities, darkening of the skin, bleeding under the skin... in clinical practice, it was seen more common in children with HbCs - SEA, HbCs and SEA mutations. – C2.delT.
- In patients with β-Thalassaemia, the manifestation of Thalassemia was quite clear in all mutants.

Table 3.13. Hematological characteristics according to the number of α-Thalassaemia mutant genes (n=27).

Hematological	1 gene $\bar{X} \pm SD$	2 genes $\bar{X} \pm SD$	3 genes $\bar{X} \pm SD$
HC (T/L)	5,14±0,41	4,70±1,12	4,29±0,96
Hb (g/dl)	93,8±10,4	92,4±13,5	87,2±8,5
Hct (%)	30,6±3,5	29,5±4,0	29,4±2,8
MCV(fL)	60,4±1,7	64,7±10,8	67,7±7,8
MCH (pg)	18,6±1,7	20,3±4,1	20,7±2,9

Comments: The quantity of hemoglobin and hematocrit decreased in all mutant forms. All disease groups had MCV expression less than 80fl, MCH decreased below 27pg

Table 3.14. HST electrophoresis characteristics according to the number of mutant genes (n=14)

Hemoglobin according	3 genes $\bar{X} \pm SD$
HbH	11,4±5,5
HbA1	83,9±14,8
HbA2	1,7±0,3

Comments: Among 27 α-Thalassaemia patients, 14 patients had the damage of 3 genes, showed the decrease of HbA2, and all had HbH.

Table 3.15. Hematological characteristics according to gene mutation in β-Thalassaemia (n=56)

Hematological characteristics	$\beta^0\beta^0$	$\beta^0\beta^E$	$\beta^0\beta$
	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$
HC (T/L)	2,56±0,52	3,53±0,56	5,01±1,1
Hb (g/dL)	68,2±12,5	79,8±14,2	101,7±15,2
Hct (%)	19,2±4,8	25,2±4,2	32,2±5,1
MCV (fL)	78,4±7,5	71,1±7,3	65,1±8,07
MCH (pg)	26,8±2,7	22,9±2,7	20,7±2,9

Comments: The number of red blood cells, the quantity of hemoglobin and hematocrit was all reduced in all mutant types. All pediatric patients had MCV less than 80 fl, MCH decreased less than 27 pg.

Table 3.16. The electrophoresis characteristics of hemoglobin according to gene mutation in β-Thalassaemia (n=56)

Hemoglobinaccording	$\beta^0\beta^0$	$\beta^0\beta^E$	$\beta^0\beta$
	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$
HbA1	35,7±32,2	26,1±24,1	73,7±26,5
HbA2	2,6±1,0	3,7±2,7	3,8±1,5
HbF	60,8±33,7	38,5±14,6	21,2±24,3
HbE		38,3±15,7	

Comments: HbA1 rate decreased much, HbA2 was normal or slightly increased, HbF appeared at a high rate. Patients with β0βE had HbE.

3.3.2 Initially building a number of pedigrees of Thalassemia children at Hai Phong Children's Hospital

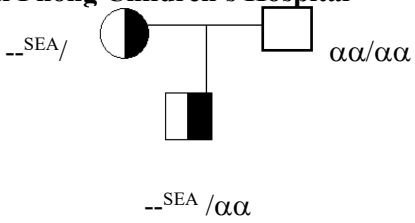

Figure 3.1. Family genealogy number 1

• Father: Vuong Duc H. 32 years old: Negative

• Mother: Tran Thi Ng. 29 years old: Carrying the SEA mutation, carrying 2 genes on one chromosome.

• Child: Vuong Tran Thien A. 4 years old: Receiving SEA mutation from mother.

Comments:
- Genotype: SEA . mutation
- Mutant combination: --SEA / αα
- Disease form: α-thalassemia

Mutations in the next generation were inherited. No new mutations have been recorded in the pedigree. Recording gene transmission to the next generation.

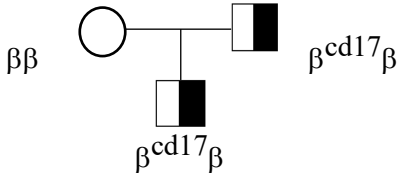

Figure 3.2. Family genealogy number 2

• Father: Hoang Sy Th. 38 years old: Carrying mutations at CD 17 of heterozygous genotype.
• Mother: Pham Thi Thuy Ph. 31 years old: negative
• Child: Hoang Nhat M. 5 months: Receiving mutation at CD 17. Carrying the mutations of Heterozygous genotype at CD 17.

Comments:
- Genotype: β0β
- Mutant combination type: βcd17β
- Disease form: β-thalassemia single heterozygote

Mutations in the next generation were inherited. No new mutations have been recorded in the pedigree. Recording gene transmission to the next generation.

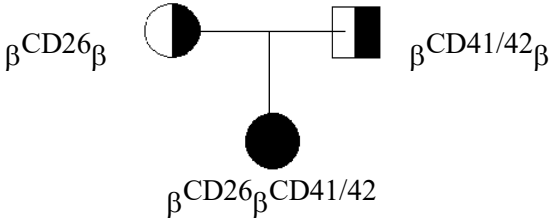

Figure 3.3. Family genealogy number 3

• Mother: Nguyen Thi H. Born 1986: Carrying mutant heterozygous at CD 26 point
• Father: Pham Duc B. Born 1984: Carrying mutant heterozygous at CD 41/42.

• Child: Pham Thao A. 12 years old: Carrying double mutant heterozygote of both parents with severe clinical expression and blood transfusion dependence.

Comment:

- Genotype: β0β; β0βE; βEβ
- Mutant combination type: $\beta^{cd41/41}\beta$; $\beta^{cd41/41}\beta^{cd26}$; $\beta^{cd26}\beta$
- Disease form: single heterozygote; combined heterozygous for HbE and HbE

Mutations in the next generation were inherited. No new mutations have been recorded in the pedigree. Recording gene transmission to the next generation.

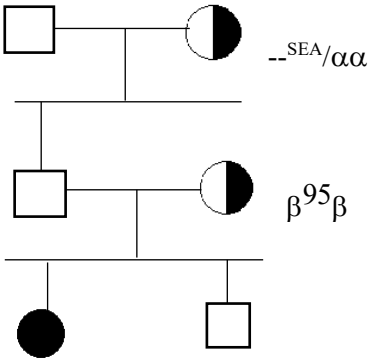

Figure 3. 4. Family genealogy number 4

• Grandfather: No mutated gene
• Grandma: Carrying the SEA mutant
• Father: not carrying a mutated gene
• Mother: Carrying beta mutation at codon 95
• Son: No mutated gene
• Daughter: Receiving a CD95 mutated chromosome from her mother. The remaining mutation on the other chromosome was suspected to be a CD95 deletion mutation (undetermined broken segment) – suspected rare mutation.

Comments:

- Genotype: SEA, β0β and β0β0 . mutations
- Mutation combination: --SEA / αα; βcd95β and βcd95βcd95
- Disease form: α-Thalassaemia; β-Thalassaemia single heterozygotss; β-Thalassaemia homozygous.

Mutations in the next generation were partly inherited. Suspecting the appearance of a new mutation in the pedigree. Recording gene transmission to the next generation.

Chapter 4. DISCUSSION

4.1. Identifying genetic mutations causing Thalassemia in pediatric patients at Hai Phong Children's Hospital

4.1.1. Clinical epidemiological features.

The mean age of hospitalization of Thalassemia patients was 6.93±5.0 years old. Thalassemia children were diagnosed with the disease with an average age of 1.86 ± 2.52 years, the youngest child with the disease was diagnosed at 2 months old, the oldest was 13 years old. Studies have shown that children with Thalassemia are often detected early with a mean age of less than 2 years. There were 83.1% of our patients who were admitted to hospitalization were between the ages of 1 - <15 years old. For pediatric α-thalassemia patients who were admitted to hospitalization there was no big difference between age groups, while 66.0% of β-thalassemia patients who were admitted to hospitalization were mainly seen in 2 age groups, 5 - 10 years old and 10-< 15 years old. Most Thalassemia patients were diagnosed at the age of <5 years, accounting for 85.6%. Studies have shown that most of the more severe forms of the disease tend to be detected earlier because of the obvious and severe clinical manifestations.

The ratio of male and female patients in the study was equal at 1.075/1. Thalassemia is a genetic disorder of autosomes with no theoretical gender difference. In our study and previous studies, both are consistent with the genetic characteristics of the disease Thalassemia, are not related to sex. Most Thalassemia patients live in rural areas.

4.1.2. Identifying genetic mutations causing Thalassemia in pediatric patients at Hai Phong Children's Hospital.

Among 83 Thalassemia children participating in the study, there were 56 β-Thalassaemia patients and 27 α-Thalassaemia patients.

We found that 23/27 α-Thalassaemia patients had SEA gene mutation, equivalent to 56.1% SEA allele mutation. The SEA mutation is the mutation of the loss of two genes on the same chromosome, which is common in Southeast Asia. In our study, the proportion of patients with 3.7 mutations was 14.8%. According to statistics, 3.7 mutation is the most common type of one-gene deletion mutation. Mainly our α-Thalassaemia patients carried mutations of HbCs – SEA and SEA, accounting for 66.6%.

Our study found that mutated alleles in β-thalassaemia showed 6 types of mutations with the rate of CD26 (39.8%), CD41/42 (25.3%), CD71/ 72 (15.7%), CD17 (15.1%), CD95 (3.6%) and IVS I-1 (1.2%). The above

mutations are also the most common mutations in Southeast Asia in general and Vietnam in particular.

In this study, the homozygous genotype for β0β0 (CD41/42 – CD41/42 and CD71/72 – CD71/72) accounted for 8.6%, the genotype was heterozygous for 2 β0β0 mutations (CD41/42 – CD17). ; CD17 – CD71/72; CD17 – IVS I-1 and CD17 – CD95) accounted for 6.9%. The HbE combined heterozygous genotype (β0βE) accounted for 44.8% and the single heterozygous genotype (β0β) accounted for 39.7%. Thus, with a high rate of mutations causing β0-thalassaemia, when the study subject has a combination of these two mutations, it will cause β-thalassaemia disease with disease symptoms from moderate to severe.

We found that genetic mutations of β-Thalassaemia patients detected in many gene positions, the most common one was in 2 exons (exon 1 + exon 2) accounting for 42.9%, exon 1 accounted for 32.1%, exon 2 accounted for 42.9%. 23.2%, intron 1 + exon 1 accounted for 1.8%. There were no cases of mutations in the promoter region. Thus, most mutations occured in the exon region, more than in the intron and promoter region. When conducting the analysis by function of the mutated gene, we found that 97.6% of the mutations were related to the RNA code translation phase. In addition, only 1 case at the RNA completion stage (1.2%), had a rare mutation (a deletion mutation at the CD95 position) and there were no mutations at the transcription stage.

4.2. Genotypic and phenotype comparison of Thalassemia pediatric patients at Hai Phong Children's Hospital

The majority of α-Thalassaemia patients were hospitalized because of jaundice while the majority of β-Thalassaemia patients came to the hospital because of symptoms of anemia. Thus, in general, the manifestation of anemia in children with β-thalassemia is more aggressive and severe than in children with α-thalassemia

A high percentage of children had symptoms of anemia at the time of admission (61.4%), especially children with β-Thalassaemia (71.4%). Signs of anemia such as blue skin, pale mucous membranes were very common in children with Thalassemia, especially in children with β-Thalassaemia. There was a statistically significant difference in the proportion of children showing anemia between the two groups of α-Thalassaemia and β-Thalassaemia.

Darkening of the skin is a manifestation of prolonged iron infection, which is common in moderate to severe thalassemia. Most of these signs appear in children with β-Thalassaemia. We studied that there was a statistically

significant difference in skin pigmentation symptoms between the two groups α-Thalassaemia and β-Thalassaemia ($p<0.05$).

Our study found that 55.4% of Thalassemia patients had splenomegaly. When comparing the two groups of subjects, we found that the percentage of children with β-Thalassaemia having splenectomy was much higher than the children in the other group. 69.6% of β-Thalassaemia patients had hepatomegaly and 66.3% of α-Thalassaemia patients had this manifestation. 45.8% of Thalassemia patients had Thalassemia expression and were more common in β-Thalassaemia patients than in α-Thalassaemia patients with this difference having statistical significance ($p<0.05$).

The majority of patients with β-Thalassaemia began to need blood transfusion at the age of < 3 years (64.7%), in which mainly patients started blood transfusion before 1 year old (37.7%). On the other hand, the majority of α-Thalassaemia patients started to need blood transfusion at the age of >3 years old, most concentrated in the age group of 3 -<5 years old. Besides very early blood transfusion, patients with β-Thalassaemia also had to have blood transfusions with a higher frequency than patients with α-Thalassaemia. Most patients with β-Thalassaemia required blood transfusion >5 times/year, accounting for 91.9%.

According to the research results, we found that most children with Thalassemia had mild to severe anemia. In the group of children with α-Thalassaemia, the average hemoglobin level of children was 90.5±11.2g/l. In the group of children with β-Thalassaemia, the average hemoglobin was 86.2±19.1g/l. The average erythrocyte quantity of Thalassemia was within normal limits (α-Thalassaemia is 4.6±0.9T/l) or tended to decrease slightly (β-Thalassaemia is 3.9±1.2T/l) . The hematocrit concentration also decreased, with an average of 27.7 ± 5.8%. The mean Hct of children with β-Thalassaemia (26.9 ± 6.5%) decreased more than that of children with α-Thalassaemia (29.6 ± 3.4%). The difference in erythrocyte quantity and hematocrit concentration between the two groups was statistically significant ($p<0.05$).

In our study, the average volume of erythrocyte was 68.5±9.0 fL, the quantity of mean hemoglobin MCH was 22.4±4.9pg. Thus, most Thalassemia patients admitted to the hospitalization had small erythrocytes and often had hypochromic erythrocytes. This was an early sign in Thalassemia screening, especially significant in patients with no clinical manifestations such as Thalassemia mild form. The quantity of ferritin serum was quite high, averaging 300 ng/dl. The average quantity of ferritin serum in β-thalassemia patients was 1067.5ng/dl and higher than in α-thalassemia patients at 213ng/ml.

4.3. Clinical and subclinical manifestations according to mutant genotype and initially building a number of pedigrees of Thalassemia children at Hai Phong Children's Hospital

Patients with α-Thalassaemia

Most α-Thalassaemia patients had little symptoms of chronic hemolytic syndrome, hepatosplenomegaly, bone deformities… because most of the α-Thalassaemia children in the study had mild symptoms.

Most patients with α-Thalassaemia had erythrocyte quantity within the normal limits. Hemoglobin and hematocrit concentration were reduced in all mutants, although there was variation between the disease forms. Patients with 1-gene and 2-gene damages had an average hemoglobin concentration of 93.8±10.4g/l and 92.4±13.5g/l, respectively. While patients carrying 3 genes of mutation had moderate anemia (average Hb is 87.2 ± 8.5g/l). Hematocrit concentration also tended to decrease in all patients.

All α-Thalassaemia patient groups in our study had expression of MCV <80fl and MCH <27pg. Specifically, The average MCV of the single gene vulnerable group was 60.4 ± 1.7fL; group of 2 mutant genes was 64.7 ± 10.8fL and group of 3 mutant genes were 67.7 ± 7.8fL. All mean MCV values were < 80fL. MCH values of α-Thalassaemia pediatric patient group had 1 vulnerable gene (18.6±1.7pg), 2 genes (20.3±4.1pg) and 3 genes (20.7±2.9pg). Thus, most of our α-Thalassaemia patients had severe hypochromic small erythrocyte.

β-Thalassaemia patients

The clinical manifestations of β-Thalassaemia are rich and varied, clearly expressed in all mutants. Most children carrying mutations of β0β0 and β0βE have typical clinical manifestations of Thalassemia, such as jaundice, anemia, bone deformity, Thalassemia face, skin tan, pale gingival mucosa, hepatomegaly , splenomegaly or splenectomy. Patients carrying the β0β single heterozygous mutation have less clear expression than the other mutants.

Hematological indices were reduced in all mutant types. All patients with β-Thalassaemia had anemia from moderate (mean hemoglobin concentration β0β0 68.2±12.5 g/l and β0βE 79.8±14.2 g/l) to mild (β0β form is 79.8±14.2 g/l) 101.7±15.2 g/l).

Pediatric patients with β0β0 β-Thalassaemia form had more severe anemia than those of β-Thalassaemia form/HbE. The number of red blood cell decreased, the average was 3.9±1.2 T/l. The number of red blood cell of children with β0β0 β-Thalassaemia type decreased more than β0βE, respectively 2.56 ± 0.52T/l and 3.53 ± 0.56T/l.

The hematocrit rate also decreased by an average of 19.2 ± 4.8% and 25.2 ± 4.2%. The mean MCV in all children with β-Thalassaemia was 70.0±8.8Fl. When analyzing by each mutant, it was found that the MVC at single heterozygous form was the smallest with an average of 65.1±8.07fL.

Children with β-Thalassaemia/HbE had smaller MCV than children with single β-Thalassaemia by 71.1 ± 7.3fL and 78.4 ± 7.5fL, respectively. MHC < 28pg, the average was 23.4 ± 5.2pg. MCH in patients with β-Thalassaemia/HbE and β-Thalassaemia with single heterozygotes decreased equally (22.9 ± 2.7pg and 20.7 ± 2.9pg). The erythrocyte characteristics in β-Thalassaemia were many small erythrocytes, severely hypochromic erythrocytes.

Hemoglobin electrophoresis in children with β-Thalassaemia showed that the hemoglobin composition in this patient group changed a lot. For patients with β-Thalassaemia in general, HbA1 decreased much, the average was 46.6 ± 34.3% of total Hb; HbA2 increased slightly, the average was 3.6 ± 1.9% and especially the rate of HbF increased very high, the average was 37.5 ± 25.7%.

With β0β0 form, HbA1 decreased to only 35.7 ± 32.2% and HbF increased very high, an average of 60.8 ± 33.7%. β0βE form, HbA1 also decreased much, the average was 26.1 ± 24.1%; HbF also increased very high, the average was 38.5 ± 14.6%; HbA2 increased slightly (3.7 ± 2.7%). Especially, there was the presence of many HbE, the average is 38.3 ± 15.7%. In the β0β group, HbA1 decreased less than the above two groups (the average was 73.7 ± 26.5%); HbA2 increased slightly (3.8 ± 1.5%) and HbF increased (the average was 21.2 ± 24.3%).

4.3.2 Genetic pedigree of some Thalassemia patients in the study

In 12 pedigree trees built, we found that most of the members belonged to the 2nd generation, only 1 case was in the 3rd generation. The results showed that, out of a total of 45 individuals participating in the pedigree study, the rate of carriers of the disease gene was 77.8% and 100% of the children carrying the disease or having the gene had father or mother or both carried the Thalassemia gene. In particular, the discovery of a mutation in the next generation was partly due to heredity and the appearance of a new mutation in the pedigree.

SOME LIMITATIONS OF THE THEME

Research sample size is small (due to funding problems and molecular biology techniques are not really popular).

Gene sequencing is not performed in all patients and their relatives

The number of pediatric patients and their family members participating in genealogical research is limited

CONCLUSION

1. Genetic mutations cause Thalassemia in pediatric patients at Hai Phong Children's Hospital.

Most children with α-Thalassaemia carried mutations in SEA (33.3%), HbCs-SEA (33.3%) and 3.7-SEA (11.2%). Among 41 α-Thalassaemia mutant alleles, SEA accounted for 56.1%.

56 β-Thalassaemia children were detected 6 mutations: CD26 (39.8%), CD41/42 (25.3%), CD71/72 (15.7%), CD17 (15.1%), CD95 (3.6%) and IVS I-1 (1.2%).

Most children with β-Thalassaemia carried mutations β0βE (44.8%) and β0β (39.7%), at least β0β0 mutation (15.5%).

HBB mutations in many gene positions, mainly mutations in 2 exons (42.9%), exon 1 (32.1%) and exon 2 (23.2%). There were no promoter region mutations. Mainly mutations related to the RNA translation stage (97.6%).

2. Genotypic and phenotype comparison of Thalassemia pediatric patients at Hai Phong Children's Hospital

Children with α-Thalassaemia were hospitalized for jaundice (63%) and children with β-Thalassaemia were hospitalized for anemia (67.9%). On hospitalization admission, most children had enlarged liver (69.6%), anemia (61.4%), splenomegaly (55.4%), jaundice (45.8%), face Thalassemia (45, 8%). Children with β-Thalassaemia (91.9% blood transfusion >5 times/year) had more blood transfusions than α-Thalassaemia (81.5% independent of blood transfusion).

The mean Hb concentration of α-Thalassaemia (90.5±11.2g/l) was higher than that of β-Thalassaemia (86.2±19.1g/l). Usually Thalassemi children had small, hypochromic red blood cells, irregular red blood cell sizes, very different sizes. The serum ferritin concentration of β-Thalassaemia children (1067.5ng/dl) was higher than that of α-Thalassaemia (213ng/ml). The average serum Fe content was 20.0±12.4 ng/ml.

3.Clinical and subclinical manifestations according to mutant genotype and initially building a number of pedigrees of Thalassemia children at Hai Phong Children's Hospital.

Clinical manifestations of α-Thalassaemia were seen in children carrying mutations of HbCs-SEA, HbCs and SEA-C2.delT. Children with α-Thalassaemia had anemia (mean Hb of mutation of 1 gene was 93.8±10.4g/l; 2 genes was 92.4±13.5g/l and 3 genes was 87.2±8.5g/l) l). The rate of HbA1 decreased slightly (83.9 - 94.2%), HbH increased (7.8±2.5% to 11.6±7.3%).

Clinical manifestations in children β-Thalassaemia in β0β0 (100%) and β0βE (76.6% - 96.2%) was less than in β0β form (9.5% - 33.3%). Children with β-Thalassaemia had anemia from moderate (mean Hb of β0β0 is 68.2±12.5g/l and β0βE is 79.8±14.2g/l) to mild (101.7±15.2 g/l). Hb composition changed: β0β0, HbA1 decreased (35.7±12.2%), HbF increased (60.8±23.7%); The β0βE form has decreased HbA1 (26.1±14.1%); HbF increased (38.5±14.6%) and HbE increased (38.3±15.7%); β0β mutant, with reduced HbA1 (73.7±26.5%); HbA2 increased (3.8±1.5%), HbF increased (21.2±4.3%).

12 families participated in genealogical research with 11 pedigrees of 2 generations and 1 pedigree of 3 generations. Thalassemia is inherited according to the rule of recessive alleles on autosomes, most mutations of the next generation are inherited from the previous generation. There was a pedigree showing the appearance of a new mutation in the offspring. This individual received 1 Thalassemia mutant gene from the mother and 1 deletion mutation from the father (rare mutation). Genealogical research is a basic and effective method, contributing to the monitoring and management of genetic resources of genetic diseases

RECOMMENDATIONS

1, Conducting research on a larger sample size

2, All gene carriers and disease carriers should be included in genetic management.

3 Conducting broader screening with basic tests to detect gene carriers.

4. Application of molecular biology techniques for large-scale screening to detect and manage disease genetic resources in the community

LIST OF SCIENTIFIC WORKS FOR PUBLICATION RELATED TO THE THESIS

1, Do Thi Quynh Mai, Nguyen Ngoc Sang, Bach Thi Nhu Quynh (2022). Clinical and hematological characteristics according to mutant genes of Thalassemia pediatric patients at Hai Phong Children's Hospital. Journal of Vietnamese Medicine, volume 509, issue 1, 342 – 347.

2, Do Thi Quynh Mai, Nguyen Ngoc Sang, Bach Thi Nhu Quynh (2022). Identification of genetic mutations causing Thalassemia in children at Hai Phong Children's Hospital. Journal of Vietnamese Medicine, volume 509, issue 1, 361 – 365