

Survey of hemoglobinopathies in Central Vietnam: Identification of a novel β -globin gene mutation (HBB:c.-122T>A).

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The Survey

The thalassemias, together with sickle cell disease (HBB: c. 20A>T), are the world's most common form of inherited anemia. The myriad manifestations of thalassemia result from the imbalanced synthesis of α -like and non- α -like globin chains and from the accumulation of unpaired counterpart.

Unpaired globin chains are unstable: they form intracellular aggregates which are insoluble and precipitate causing decreased deformability, membrane damage and selective removal of the damaged cell. Ineffective erythropoiesis and shortened red cell survival will lead to chronic anemia.

The β -thalassemias are characterized by a quantitative deficiency of β -globin chains underlain by a striking heterogeneity of molecular defects. Mutations that completely inactivate the β gene resulting in no β -globin production cause β^0 -thalassemia. Other mutations allow the production of some β globin but in markedly decreased amounts, and are classified as β^+ -thalassemias.

According to the severity of the phenotype, β -thalassemias can be subclassified in three different groups: β -thalassemia minor (Tm), β -thalassemia intermedia (TI) and β -thalassemia major (TM). Carriers for Tm, which are heterozygotes for a defective β -gene, show microcytic and hypochromic red cells with or without anemia. Inheritance of two β^0 -thalassaemia alleles, homozygous or compound heterozygous states, results in TM with ineffective erythropoiesis, peripheral hemolysis and subsequent transfusion-dependent anemia. Homozygotes $\beta^+\beta^+$ or compound heterozygotes $\beta^+\beta^0$ are usually affected with TI and show a moderate to severe, partially compensated, hemolytic anemia which does not or only occasionally requires transfusions [1].

After 60 years of significant progress, a supportive care including transfusions and iron chelation is still the main treatment option for most thalassemia patients. Hematopoietic stem cell transplantation, not feasible for most patients, is currently the only definitive cure for β -thalassemia. Approximately 80% of the annual births of babies with severe conditions occur in developing and low-income countries, many of which have extremely limited healthcare resources. Therefore, thalassemia carrier screening programs are essential to reduce the impact of disease.

Routine diagnosis of β -thalassemia trait is based on microcytic parameters and/or elevated levels of Hb A2 (≥ 3.5) although, only molecular DNA analysis can give reliable results.

The ability to perform DNA analysis has become an increasingly important requirement and the acquisition of such skills requires the development of suitable training programs involving hematologists, pediatricians, biologists and technical staff. A start has been made in developing and low-income countries but there is much more to be done including an efficient implementation of partnerships supporting countries in acquiring and developing specific skills.

In this report we summarize results obtained during a screening program for hemoglobinopathies (Hbpathies) performed in the context of a cooperation between the Universities of Hue, Vietnam, and Sassari, Italy.

In Vietnam, both β -thalassemia and Hb E (HBB: c.79G>A) are prevalent and represent an important cause of childhood chronic disease [2]. The first study for β -thalassemia was performed in the North of Vietnam [3]. Subsequent analysis has been carried out in the South [2, 4]. More recent surveys were conducted in the central area of the country [5, 6, 7]. Although several studies have been performed, epidemiological data is still incomplete.

To date, eight β -thalassemia mutations have been documented in Vietnam six of which are β^0 mutations and two belong to the β^+ type [2-8]. The most commons are the frameshift mutation at codons 41/42 (HBB:c.126_129delCTTT), the nonsense mutation at codon 17 (HBB:c.52A>T) and the frameshift mutation at codon 95 (HBB:c.287_288insA), which is known as the "Vietnamese" mutation. The other mutations are less frequent: the β^+ -28 (HBB: c.78A>G), the codon 26 (HBB: c. 79G>T), the IVS-I-1 (HBB:c.92+1G>T), the frameshift mutation at codons 71/72 (HBB:c.216_217insA) and the β^+ IVS-II-654 (HBB:c.316-197C>T). The latter, despite it being a β^+ mutation, leads to a transfusion-dependent phenotype in homozygous or compound heterozygous states.

The missense mutation GAG>AAG (Glu>Lys) at codon 26 of the Hb E structural variant also activates a cryptic, alternative, splice site and leads to a β^+ -thal phenotype due to a 25.0–30.0% reduction of β^E -globin synthesis. The Hb E heterozygote is mildly affected and the Hb E homozygosity is a benign disorder with a mild β -globin chain deficit which is comparable to that seen in a β^0 -thalassemia heterozygote. However, compound heterozygotes $\beta^E\beta^0$ are often severely affected.

Our study included nearly 200 individuals referred to Hue Medicine and Pharmacy College and PhuVang District Hospitals for hematological and clinical evaluation. Samples have undergone to Hbpathies screening on whole blood lysates performed by cation-exchange high-performance liquid-

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chromatography (CE-HPLC) and by isoelectric focusing [9]. Any qualitative or quantitative alteration observed in Hb profiles was further investigated by DNA analysis which included: Multiplex Ligation-Dependent Probe Amplification (MLPA) analysis of HBB and HBA cluster, PCR and Sequencing Analysis of the β -globin gene, K562 cell-based luciferase reporter assay on β -globin gene promoter [10].

A total of three known β -globin gene mutations and a novel one were observed. The three previously described were: the Hb E (HBB: c.79G>A), the mutation at codons 41/42 (HBB:c.126_129delCTTT) and the mutation at codon 17 (HBB:c.52A>T). These have been found in heterozygosity or in compound heterozygous $\beta^E\beta^0$ state. Wide phenotypic variability in $\beta^E\beta^0$ patients is described ranging from a severe condition indistinguishable from TM to a mild form similar to TI [11].

The reasons of this striking variability are not completely understood. Despite seemingly identical genotypes, patients from the same family may present substantial differences in the severity of the clinical phenotype. An ameliorating effect of Hb F levels on morbidity is reported, although the basis of increased Hb F is usually unknown. In a review of 378 patients with Hb E- β^0 -thalassemia from Thailand, the Hb levels ranged from 3 to 13 g/dl, with an average of 7.7 g/dl [11].

In our study, the average Hb level was 8.13 g/dl, with Hb F significantly increased. None of the $\beta^E\beta^0$ patients needs regular transfusions. The level of persistent Hb F synthesis appears to be the major modulator of clinical mildness.

Sequence analysis revealed the mutation T→A at position -72 (HBB:c.-122T>A) of the β -globin gene in 5-year-old child. The mutation is novel and falls within the CCAAT box. The proband presented with a mild microcytic anemia as both his father and paternal grandfather. None of them had ever required transfusions. The other family members showed normal hematological and biochemical features. Sequencing revealed that the same mutation identified in the proband's β -globin gene was also present in both father and grandfather. No alteration was observed in the HBA cluster except for a triplication highlighted in the proband's father, which does not seem to affect the phenotype. The presence of Hb Constant Spring was excluded in all carriers [10]

To date, one mutant at β -promoter, the -28 (HBB: c.78A>G), has been identified in Vietnam [2,4]. This variant occurs in the TATA box and has been reported as β^+ -thalassemic allele due to the mildness of the clinical manifestations observed in compound heterozygotes $\beta^E\beta$ -28 from Thai population [12].

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A luciferase reporter assay was used to test the impact of the -72 mutation on gene expression.

Our in vitro experiments showed that the -72 mutation reduces the transcriptional activity of the promoter by about 50% , so it can be classified as a β^+ -thalassemic allele [10]. However, neither clinical impact of mutation nor its relevance in management programs can be precisely predict since it was found in a heterozygous state. Indeed, association of β^+ -thalassemia with β^0 or β^E mutations results in a very heterogeneous clinical spectrum, ranging from asymptomatic to severe transfusion-dependent state [13].

Furthermore, it has been noted that even in the case of mild disease, β -TI patients may still suffer from many complications including endocrinopathies, osteoporosis, cardiac disease and pulmonary hypertension [14].

Genotype-to-phenotype prediction has important implications for carrier screening, prenatal diagnosis, prevention and management strategies and requires a comprehensive knowledge of the spectrum of β -thalassemia mutations, more so in countries like Vietnam where a wide genetic variability exists. It is furthermore important to emphasize that the high frequency of β -thalassemia facilitates new allele combinations, whose clinical phenotype is not known.

For these reasons, identification and functional characterization of β -thalassemic alleles, both severe and mild, are essential to reduce the burden of thalassemias and help in planning of early intervention strategies.

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