Title

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Journal

Hemoglobin, 44(1)

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Publication Date

2020

DOI

10.1080/03630269.2020.1731530

Peer reviewed

HHS Public Access

Author manuscript

Hemoglobin. Author manuscript; available in PMC 2021 March 16.

Published in final edited form as:

Hemoglobin. 2020 January ; 44(1): 1–9. doi:10.1080/03630269.2020.1731530.

Hb S/β-Thalassemia in the REDS-III Brazil Sickle Cell Disease Cohort: Clinical, Laboratory and Molecular Characteristics

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Abstract

We described the clinical, laboratory and molecular characteristics of individuals with Hb S (*HBB*: c.20A>T)/β-thalassemia (Hb S/β-thal) participating in the Recipient Epidemiology and Donor Evaluation Study (REDS-III) Brazil Sickle Cell Disease cohort. *HBB* gene sequencing was performed to genotype each β-thal mutation. Patients were classified as Hb S/β^0-thal, Hb S/β^+-thal-severe or Hb S/β^+-thal based on prior literature and databases of hemoglobin (Hb) variants. Characteristics of patients with each β-thal mutation were described and the clinical profile of patients grouped into Hb S/β^0-thal, Hb S/β^+-thal and Hb S/β^+-thal-severe were compared. Of the

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

2793 patients enrolled, 84 (3.0%) had Hb S/β^0-thal and 83 (3.0%) had Hb S/β^+-thal; 40/83 (48.2%) patients with Hb S/β^+-thal had mutations defined as severe. We identified 19 different β-thal mutations, eight Hb S/β^0-thal, three Hb S/β^+-thal-severe and eight Hb S/β^+-thal. The most frequent β^0 and β^+ mutations were codon 39 (*HBB*: c.118C>T) and IVS-I-6 (T>C) (*HBB*: c.92+6T>C), respectively. Individuals with Hb S/β^0-thal had a similar clinical and laboratory phenotype when compared to those with Hb S/β^+-thal-severe. Individuals with Hb S/β^+-thal-severe had significantly lower total Hb and Hb A levels and higher Hb S, white blood cell (WBC) count, platelets and hemolysis markers when compared to those with Hb S/β^+-thal. Likewise, individuals with Hb S/β^+-thal-severe showed a significantly higher occurrence of hospitalizations, vaso-occlusive events (VOE), acute chest syndrome (ACS), splenic sequestration, blood utilization, and hydroxyurea (HU) therapy.

Keywords

Clinical events; Hb S/β^+-thalassemia (Hb S/β^+-thal); Hb S/β^0-thalassemia (Hb S/β^0-thal); sickle cell disease; thalassemia mutation

Introduction

Sickle cell disease, caused by a point mutation on the β-globin gene (Hb S or *HBB*: c.20A>T, p.E6V), is one of the most common monogenic disorders worldwide. The clinical hallmarks of the disease, vaso-occlusion and hemolytic anemia, result in both acute complications as well as progressive organ damage, causing multisystem disease [1]. The compound heterozygous state for the Hb S allele and a β-thalassemia (β-thal) mutation (Hb S/β-thal), is associated with considerable variation in clinical severity [2]. The clinical phenotype primarily depends on the β-thal molecular mutation and its impact on Hb A synthesis. The complete inactivation of the *HBB* gene by a β-thal mutation that results in no normal Hb A [Hb S/β^0-thal (Hb S/β^0-thal)] is typically associated with a severe clinical presentation, similar to Hb SS (β^S/β^S). Therefore, Hb SS and Hb S/β^0-thal are sometimes classified together as sickle cell anemia [3]. Alternatively, β-thal mutations that result in reduction of Hb A (Hb S/β^+-thal) are associated with a wide spectrum of clinical severity, ranging from severe disease, similar to Hb S/β^0-thal [4], to mild disease with few clinical manifestations [5].

Although knowledge of the epidemiology, clinical and laboratory profile, genetic modifiers, and health burden of sickle cell disease is growing, Hb S/β-thal is less well characterized than other sickle cell disease genotypes. The documented clinical progression of Hb S/β-thal is derived primarily from smaller studies or cohorts established before widespread use of current clinical strategies that have improved the management of sickle cell disease, including early diagnosis through newborn screening programs, prophylactic penicillin, treatment with hydroxyurea (HU), and chronic transfusion therapy [6-10]. Therefore, Hb S/β-thal warrants further investigation to establish the clinical profile, particularly the correlation of disease severity associated with specific β-thal mutations. In one cohort of Hb S/β-thal patients from Jamaica, individuals were divided into four groups according to the amount of Hb A production. Hb S/β^0-thal for those without Hb A synthesis and three types

of Hb S/β^{+}-thal (type I: 1.0-7.0% Hb A, type II: 7.0-14.0% Hb A, and type III: 14.0-25.0% Hb A) [4]. Patients with Hb S/β^{+}-thal have also been previously classified as Hb S/β^{+}-thal or Hb S/β^{++}-thal, depending on the degree of *HBB* synthesis reduction (relatively more severe or mild phenotype, respectively). However, these classifications are not commonly used in clinical practice and other large cohorts of Hb S/β-thal populations to define severity are lacking [2,11]. In this study, we describe the demographic, clinical and laboratory profile of a group of patients with Hb S/β-thal in a Brazilian sickle cell disease cohort and compare disease manifestations based on the β-thal mutation.

Materials and methods

This study is part of the Brazil Component of the Recipient Epidemiology and Donor Evaluation Study (REDS-III) Sickle Cell Disease cohort [12], which included health centers in six cities in Brazil: (1) Fundação Hemominas in Belo Horizonte (HBH), Juiz de Fora (JFO), and Montes Claros (MOC), in the Minas Gerais State; (2) Fundação Hemorio in Rio de Janeiro, Rio de Janeiro State; (3) Fundação Hemope in Recife, Pernambuco State; and (4) the Child Institute at Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP) in São Paulo, São Paulo State. Participants were randomly selected as eligible from each center's patient population and recruited at scheduled clinic visits. An interview, medical record abstraction, and blood collection were performed for all consenting subjects at enrollment. The interview captured comprehensive demographics, and vital signs were assessed. Medical records were reviewed by hematologists or research coordinators under the supervision of hematologists to abstract clinical data using standardized definitions of the phenotypic manifestations of sickle cell disease [13]. Transfusion history and blood bank data, including red blood cell (RBC) phenotype and antibody data, were extracted from each center's database. A comprehensive electronic database was created to centralize all demographic, clinical, laboratory and transfusion information. Participants were prospectively followed and monitored for the occurrence of clinical manifestations during three study visits [12]. This manuscript included retrospective data from the participants' entire life, from birth to the day that medical record was reviewed during the enrollment to the cohort (from November 2013 to March 2015), as well as prospective data collected during the first follow-up and second follow-up visits (from November 2014 to August 2018).

Whole blood was collected from all participants. DNA was extracted from peripheral white blood cells (WBCs) using the alcohol precipitation method and quantified using real-time polymerase chain reaction (PCR). The sickle cell disease genotype was confirmed for all participants by allele-specific pyrosequencing [14]. Participants were initially screened with the pyrosequencing technique based on Qiagen technology (Qiagen GmbH, Hilden, Germany). If the pyrosequencing result did not match the diagnosis recorded in the medical files or heterozygous Hb S was detected, Sanger sequencing of the *HBB* gene was performed by an ABI PRISM® 3500 analyzer (Applied Biosystems, Foster City, CA, USA).

Participants were phenotypically classified as having Hb S/β^{0}-thal, Hb S/β^{+}-thal or Hb S/β^{+}-thal-severe according to β-thal mutation. This classification was based on genotype-phenotype correlations that have been shown in previously published studies [4,5,7,8,15-22]

and review of Hb variant databases [23,24]. The following mutations were classified as Hb S/β^0-thal: IVS-II-849 (A>G) (*HBB*: c.316-2A>G), IVS-I-1 (G>A) (*HBB*: c.92+1G>A), codon 39 (C>T) (*HBB*: c.118C>T), IVS-II-1 (G>A) (*HBB*: c.315+1G>A), codons 106/107 (+G) (*HBB*: c.321_322insG), IVS-I-2 (T>C) (*HBB*: c.92+2T>C), codon 6 (−A) (*HBB*: c.20delA), IVS-I-2 (T>G) (*HBB*: c.92+2T>G). The following mutations were classified as Hb S/β^+-thal-severe: IVS-I-110 (G>A) (*HBB*: c.93-21G>A), IVS-I-5 (G>C) (*HBB*: c:92+5G>C) and IVS-I-5 (G>A) (*HBB*: c.92+5G>A). All other mutations were classified as Hb S/β^+-thal. Three patients with a β-thal mutation that could not be identified through Sanger sequencing and two patients without DNA available for genotyping, were classified according to clinical and hematological data.

The Brazilian National Ethics Committee for Research (reference #02790812.0.1001.0065), local Ethics Committees, and Institutional Review Boards at participating institutions in the USA approved the study. Written informed consent was obtained from the patients and/or guardians. Age appropriate assent was also obtained for children more than 6 years of age.

Statistical Analysis

Categorical variable results are presented as frequencies, and continuous variable results as means ± standard deviations (SD), when normally distributed (assessed by the Shapiro-Wilk normality test), and median ± interquartile ranges (IQR) otherwise. Association between categorical variables, comparing Hb S/β^0-thal, Hb S/β^+-thal and Hb S/β^+-thal-severe groups, was assessed using independent I^2 tests or logistic regression modeling, including adjustment by sex and age. Association between continuous variables and Hb S/β-thal for two group comparisons were assessed by the Wilcoxon Mann-Whitney test, and for three groups by Kruskal-Wallis (with post hoc multiple comparisons by the Dunn test) or analysis of a variance (ANOVA) (with post hoc multiple comparisons by the Turkey test). Statistical analyses was conducted using R software version 3.5.1 (https://cran.r-project.org/), with significance defined as $p \leq 0.05$.

Results

Of the 2793 patients enrolled in the REDS-III Sickle Cell Disease cohort [12], 83 (3.0%) had Hb S/β^+-thal and 84 (3.0%) had Hb S/β^0-thal. Of these, 167 Hb S/β-thal patients, 93 (55.7%) were children (<18 years) and 74 (44.3%) were adults (mean age at study enrollment 18.6 ± 12.9 years); 88 (51.8%) were males and 79 (48.2%) were females. The frequency of Hb S/β^+-thal and Hb S/β^0-thal varied between the six REDS-III Brazil clinical sites, with the highest frequencies observed in Hemope and Hemominas in JFO, respectively (Table 1). The majority of Hb S/β^0-thal participants reported mixed race ($n = 45$; 53.6%), followed by Caucasian ($n = 20$; 23.8%), Black ($n = 16$; 19.0%) and unknown ($n = 3$; 3.6%). Most participants with Hb S/β^+-thal reported mixed race ($n = 49$; 59.0%), followed by Caucasian ($n = 19$; 22.9%), Black ($n = 14$; 16.9%) and unknown ($n = 1$; 1.2%). Of 83 individuals, 43 (51.8%) were classified as Hb S/β^+-thal and 40 (48.2%) as Hb S/β^+-thal-severe.

We identified eight β^0-thal and 11 β^+-thal mutations in the study. Of 83 individuals with Hb S/β^+-thal, 26 (31.3%) carried the following mutations: IVS-I-6 (T>C) (*HBB*: c.92+6T>C), 17 (20.5%) IVS-I-110 and 16 (19.3%) IVS-I-5. Of 84 individuals with Hb S/β^0-thal, 46 (54.8%) carried the codon 39 and 21 (25.0%) the IVS-I-1 (G>A) mutations. The frequency of less common mutations and distribution of mutations in the six REDS-III sites is shown in Table 1. The mutations found in the REDS-III participants can be classified into seven groups according to their effect on gene function and Hb A production: transcriptional mutations in promoter regulatory elements that decrease transcription, two categories of mutations that impact RNA translation and four categories of mutations that impact RNA processing (Figure 1).

The proportion of patients with Hb S/β^+-thal, Hb S/β^+-thal-severe and Hb S/β^0-thal varied at the six centers (Table 1). Males represented 60.5% ($n = 26$) of individuals in the Hb S/β^+-thal group compared with 42.5% ($n = 17$) and 53.6% ($n = 45$) in the Hb S/β^+-thal-severe and Hb S/β^0-thal groups, respectively ($p = 0.25$). The median age varied between the groups, but not significantly: Hb S/β^+-thal-severe (17 ± 15.5), followed by Hb S/β^0-thal (16.5 ± 17.3) and Hb S/β^+-thal (11 ± 23.5) ($p > 0.05$).

Individuals with Hb S/β^+-thal-severe had significantly lower total Hb and Hb A levels and higher Hb S, WBC, platelets and hemolysis markers when compared to those with Hb S/β^+-thal. There was no significant difference in levels of total Hb, WBC, platelets, reticulocytes, and bilirubin levels between individuals with Hb S/β^+-thal-severe and Hb S/β^0-thal (Table 2).

Patients classified as Hb S/β^+-thal-severe showed a significantly higher occurrence of hospitalizations in the past year, vaso-occlusive events (VOE), acute chest syndrome (ACS), acute splenic sequestration, and a trend towards higher abnormal transcranial Doppler (TCD) when compared to those with Hb S/β^+-thal based on the adjusted logistic regression models controlled for age and sex between the groups (Table 3). With the exception of abnormal TDC, which was significantly more frequent in the Hb S/β^+-thal-severe group, there was no difference in the occurrence of clinical manifestations in patients with Hb S/β^+-thal-severe and Hb S/β^0-thal. Hydroxyurea therapy, blood transfusions (both lifetime history and transfusions in the last 12 months), splenectomy, and cholecystectomy were more common for patients with Hb S/β^+-thal-severe and Hb S/β^0-thal when compared with those with Hb S/β^+-thal based on the adjusted logistic regression models controlled for age and sex between the groups.

Additional descriptive laboratory, clinical and management data of individuals with Hb S/β-thal, according to each molecular mutation classification, are shown in the supplemental online material (Supplementary Tables 1 and 2).

Discussion

In this study, we characterized the clinical and laboratory profile and identified β-thal mutations in a cohort of sickle cell disease patients with Hb S/β-thal from Brazil, as well as examined the association of these mutations with clinical outcomes. The clinical profile of

our cohort does not support the concept that all Hb S/β^+-thal patients have a milder disease phenotype than other genotypes of sickle cell disease. Instead, we demonstrated a varying clinical presentation with almost half of the Hb S/β^+-thal patients showing a severe presentation of sickle cell disease, similar to those with Hb S/β^0-thal. Our data suggests that patients with Hb S/β^+-thal-severe, should be treated in the same manner as those with Hb S/β^0-thal, given their similar laboratory profile and clinical outcomes. Hb S/β^+-thal patients with severe disease have previously been shown to be phenotypically similar to Hb S/β^0-thal [4]. In our cohort, 124 (74.3%) of 167 participants carried were Hb S/β^+-thal-severe or Hb S/β^0-thal, showing that Hb S/β-thal is a subtype of sickle cell disease with increased clinical severity in Brazil compared to reports from other countries [4,8].

More than 300 unique β-thal mutations have been reported so far worldwide, about 35 of which have been reported in Brazil [5,25-34]. Overall, 19 different β-thal mutations were identified on 163 alleles in this study. The three most frequent mutations [codon 39, IVS-I-6 and IVS-I-1 (G>A)] represented 55.7% of β-thal mutations in this cohort. The Portuguese type (IVS-I-6) mutation was the most frequent β^+-thal mutation, reflecting the Portuguese colonization of Brazil between the 16th and 19th centuries. The codon 39 mutation was the most frequent β^0-thal mutation in this study. This is thought to be the most common β-thal mutation reported in the Mediterranean area and also in Brazil [25-33]. It is a common mutation in Italian [35,36], Spanish [37,38], German [39] and Portuguese [40] populations. Immigration from all of these countries have contributed to the high frequency of this allele in Brazil, during and after the colonization period. The diversity of β-thal mutations in REDS-III participants reflects the diverse ancestry and admixture of the Brazilian population. The most common self-defined race in the REDS-III cohort was mixed race, including in the subset of patients with Hb S/β-thal. However, the Caucasian race was more common in patients with Hb S/β-thal than in the REDS-III overall sickle cell disease cohort [12], suggesting an important contribution of thalassemia mutations from Caucasian immigrants.

Overall, individuals with Hb S/β^+-thal in this cohort demonstrated lower hemolytic anemia than those with Hb S/β^0-thal, and WBC and platelets were more often within normal clinical reference values. This is consistent with a previous report of laboratory data collected from Hb S/β-thal patients participating in the USA Cooperative Study of Sickle Cell Disease (CSSCD) [41]. A study conducted in Brazil also demonstrated that Hb S/β^0-thal patients have a higher degree of laboratory indices of severity when compared with Hb S/β^+-thal patients [42]. Hb S/β-thal severity typically depends on the β-thal mutation that defines the amount of normal Hb A produced. Participants with Hb S/β^+-thal-severe had lower levels of Hb A, higher degree of hemolytic anemia and higher levels of WBC and platelets than those with Hb S/β^+-thal, consistent with data reported by other investigators [4,6,8,10,43]. Hb F levels were variable in our cohort, but were higher in Hb S/β^+-thal-severe and Hb S/β^0-thal patients. However, evaluation of possible quantitative differences according to mutations is difficult because of the influence of HU therapy, the wide variability in age and possibility that increased severity may represent an up-regulation factor for higher Hb F expression [44,45].

As previously descried [4,11], patients with Hb S/β^0-thal in our study had a more severe disease course than those with Hb S/β^+-thal. Vaso-occlusive crises followed by ACS and acute splenic sequestration were the most common clinical complications observed. Our overall prevalence of ACS in Hb S/β^+-thal is similar to that observed in patients from another Brazilian sickle cell disease cohort (8/15; 53.3%) [42]. However, ACS was only reported in 18.5% of Hb S/β^+-thal participants in the CSSCD cohort [46,47]. Even comparing patients with the same molecular mutation, our ACS proportions tended to be higher than observed in patients from a Jamaican cohort. For example, the prevalence of ACS in the Jamaican cohort *vs.* our cohort for mutations IVS-I-5 (G>C), −29 (A>G) (*HBB*: c.−79A>G), polyadenylation signal A (polyA) site (*HBB*: c.*110T>C) and −88 (C>T) (*HBB*: c.−138C>T), was 38.0 *vs.* 75.0%, 16.0 *vs.* 44.4%, 22.0 *vs.* 50.0% and 9.0 *vs.* 50.0%, respectively [4]. Although these comparisons should be interpreted with caution, due to variation in study design and case definitions, these differences may show that the clinical presentation of Hb S/β^+-thal is affected by other factors, such as known genetic modifiers including coinheritance of α-thalassemia (α-thal) and Hb F levels, but also possibly unknown genetic factors, as well as regional differences such as treatment with HU or chronic transfusions, and potentially, local environmental factors that are incompletely understood.

In our cohort, individuals with Hb S/β^+-thal-severe and Hb S/β^0-thal had a high occurrence of acute splenic sequestration and splenectomy. Previously published data show that persistence of splenomegaly and splenectomy is more common in patients with Hb S/β^0-thal and Hb S/β^+-thal type II compared to those with Hb SS [8]. These data indicate that parental education to recognize signs and symptoms of acute splenic sequestration may avoid premature deaths in this subset of sickle cell disease patients.

The clinical manifestations that result from cumulative organ damage were rare in the Hb S/β^+-thal patients. However, our data show that avascular necrosis is not necessarily more common in Hb S/β^+-thal-severe and Hb S/β^0-thal groups. These data suggest that regardless of severity, all patients with Hb S/β^+-thal should be monitored for the occurrence of avascular necrosis. Our high prevalence of VOE (69.8%) in Hb S/β^+-thal patients also confirms that patients with genotypes typically classified as less severe, nevertheless have a high occurrence of pain crises [48].

In contrast to the high occurrence of ischemic stroke in the Hb S/β^0-thal patients, our data show a significantly higher risk of abnormal TCD in the Hb S/β^+-thal-severe when compared to Hb S/β^0-thal. Incidence rates of cerebrovascular accident in patients with Hb S/β^+-thal was slightly higher than those with Hb S/β^0-thal in the CSSCD cohort [49]. These data indicate that at least some Hb S/β^+-thal patients are at risk for stroke. Perhaps the absence of stroke in our Hb S/β^+-thal cohort can be explained, at least in part, by the high use of HU in the Hb S/β^+-thal-severe group, as HU decreases the TCD velocities [50] and the risk of stroke [51]. Although TCD screening is not recommended for children with Hb S/β^+-thal according to current protocols [52,53], our data suggest that it may be beneficial, especially in those patients with Hb S/β^+-thal-severe.

Transfusion therapy has previously been shown to be significantly more frequent in patients with Hb SS and Hb S/β^0-thal than in those with Hb S/β^+-thal [54]. Though no Hb S/β^+-thal patients and only one Hb S/β^0-thal patient in our cohort were treated with chronic transfusion therapy, our data demonstrated relatively high utilization of blood transfusions to manage acute complications of Hb S/β-thal. These data corroborate our observation that patients with Hb S/β^+-thal-severe and Hb S/β^0-thal are significantly affected by acute complications of sickle cell disease. Hydroxyurea therapy was more commonly used in patients with Hb S/β^+-thal-severe and Hb S/β^0-thal. Hydroxyurea has been used successfully in patients with Hb S/β^+-thal, and the response is characterized by an increase in Hb F levels and a reduction in both ACS and VOE episodes [55].

In this study we classified β-thal mutations into Hb S/β^0-thal, Hb S/β^+-thal and Hb S/β^+-thal-severe, due to the relatively small number of participants with each molecular mutation. However, this type of classification has not typically been used in clinical practice [2]. Even within each group, mainly in the Hb S/β^+-thal group, there is a wide phenotype variation. Furthermore, eligibility for enrollment for the REDS-III Sickle Cell Disease cohort did not include any criteria for severity of disease. However, patients that did not come for routine care at the clinical centers or could not be successfully contacted by study staff during the enrollment period, may represent milder forms of Hb S/β^+-thal. Ultimately, the phenotype differences between patients with Hb S/β^0-thal/Hb S/β^+-thal-severe and Hb S/β^+-thal are likely attributable to differences in disease progression for sickle cell disease, as Hb S/β^0-thal/Hb S/β^+-thal-severe groups used more treatments that reduce the frequency of certain complications of sickle cell disease, mainly HU therapy.

In summary, this study confirms that Hb S/β-thal is a heterogeneous subtype of sickle cell disease in Brazil, and the phenotype depends largely on the β-thal mutation. These molecular mutations significantly affected the clinical and laboratory presentation with the IVS-I-5 (G>C), IVS-I-5 (G>A) and IVS-I-110 β^+-thal mutations associated with a more severe phenotype, similar to Hb S/β^0-thal, while other identified mutations β^+-thal showed a less severe presentation. An important clinical lesson derived from these data may be that genotyping of the β-thal mutation is an important tool to predict the expected clinical severity of patients with Hb S/β-thal. Thus, additional genotyping could help to enhance the likelihood that patients receive the appropriate monitoring and treatment of their specific sickle cell disease and subsequent manifestations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

First, the authors acknowledge all participants and parents of pediatric participants for their cooperation in being part of this the study. The authors also thank the staff at all participating Brazilian and American research sites. Without all of these contributions, this study would not have been possible. This study was supported by the National Heart, Lung, and Blood Institute (NHLBI) Recipient Epidemiology and Donor Evaluation Study-III: International Component (Brazil), with the participation of the following centers and investigators. Brazilian participants: Instituto de Medicina Tropical de São Paulo (USP), Ester C. Sabino, Cecilia Alencar; Fundação Pró-Sangue (São Paulo), Alfredo Mendrone Jr., Cesar de Almeida Neto, Ligia Capuani; Instituto de Tratamento do Câncer Infantil (São Paulo), Miriam V. Flor-Park; Faculdade de Medicina da Universidade de São Paulo (São

Paulo), Paula Blatyta; Hemominas, Belo Horizonte (Minas Gerais), Anna Bárbara de Freitas Carneiro-Proietti, Carolina Miranda Teixeira, Tassila Salomon, Franciane Mendes de Oliveira, Valquíria Reis. Hemominas; Montes Claros (Minas Gerais), Rosemere Afonso Mota, José Wilson Sales; Hemominas, Juiz de Fora (Minas Gerais), Daniella de Oliveira Werneck Rodrigues; Fundação Hemope, Recife (Pernambuco), Paula Loureiro, Aderson Araújo, Maria do Carmo Valgueir; Hemorio, (Rio de Janeiro), Clarisse Lobo, Claudia Maximo, Luiz Amorim; Instituto de Matemática e Estatística da Universidade de São Paulo, USP (São Paulo), João Eduardo Ferreira, Márcio Katsumi Oikawa, Pedro Losco Takecian, Mina Cintho Ozahata, Rodrigo Muller de Carvalho. US Investigators: Vitlant Research Institute and University of California San Francisco (UCSF), San Francisco, CA, Brian Custer, Michael P. Busch, Shannon Kelly and Thelma Therezinha Gonçalez; Research Triangle Instiute (RTI), Rockville, MD; Donald Brambilla, Liliana R. Preiss, Christopher McClure; UCSF Benioff Children's Hospital Oakland, Oakland, CA, Carolyn Hoppe; NHLBI, National Institutes of Health (NIH), Bethesda, MD, Simone A. Glynn.

Funding

This study was supported by the National Institutes of Health, National Heart, Lung, and Blood Institute by Grant HHSN268201100007I. André R. Belisário received a scholarship from the International Traineeships in AIDS Prevention Studies program (ITAPS) to participate in a scientific manuscript writing program at the University of California, San Francisco, CA, USA.

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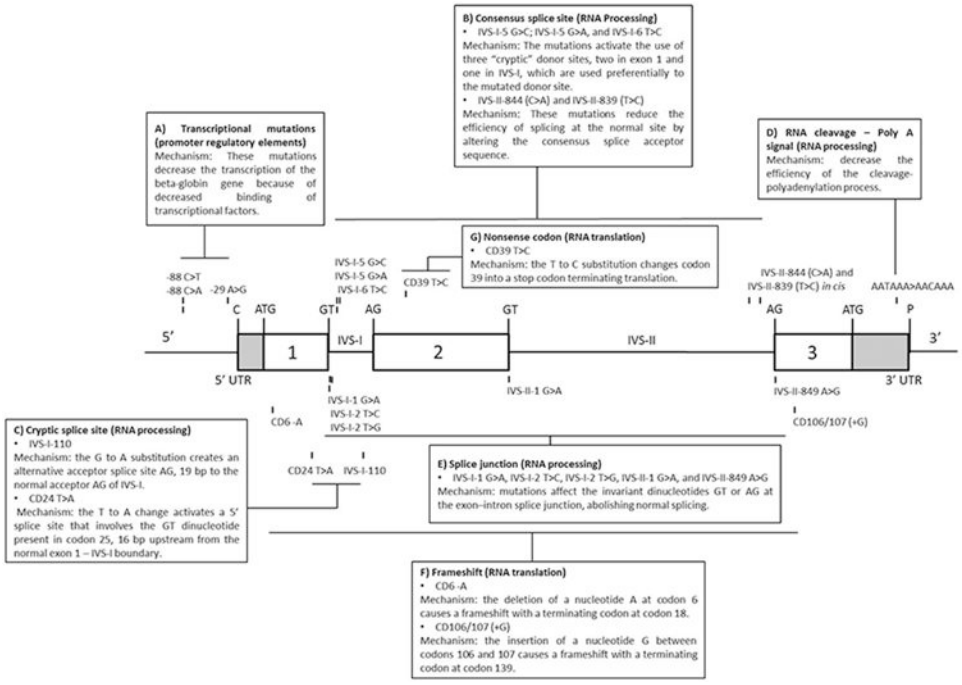

Figure 1.

Mutations causing β-thal observed in this study. The *HBB* is depicted with the three exons (1, 2 and 3), two introns (IVS-I and IVS-II), conserved sequences in the 5' and 3' untranslated regions (5'UTR and 3'UTR), and the invariant dinucleotides at the exon-intron junctions of the gene. The vertical lines above or below *HBB* represent the sites of the different point mutations. The mutations found in the study can be classified into four groups according to their effect on gene function and Hb A production: (A) transcriptional, (B) consensus splice site, (C) cryptic splice site, (D) polyA signal mutations, (E) Splice junction, (F) frameshift and (G) nonsense codon.

Table 1.

β-Thalassemia mutation distribution according to hemocenters participating in the Recipient Epidemiology and Donor Evaluation Study-III Sickle Cell Disease cohort, Brazil.

β-Thal	Total *n* (%)	HBH *n* (%)	JFO *n* (%)	MOC *n* (%)	Hemope *n* (%)	Hemorio *n* (%)	ITACI *n* (%)
Hb S/β^{+}-thal (severe)	40 (24.0)	6 (14.6)	2 (13.3)	4 (30.8)	16 (36.4)	8 (19.5)	4 (30.8)
IVS-I-110 (G>A)	17 (10.2)	3 (7.3)	1 (6.7)	3 (23.1)	3 (6.8)	5 (12.2)	2 (14.3)
IVS-I-5 (G>C)	16 (9.6)	1 (2.4)	0 (0.0)	0 (0.0)	12 (27.3)	2 (4.9)	1 (7.7)
IVS-I-5 (G>A)	7 (4.2)	2 (4.9)	1 (6.7)	1 (7.7)	1 (2.3)	1 (2.3)	1 (7.7)
Hb S/β^{+}-thal	43 (25.7)	11 (26.8)	2 (13.3)	0 (0.0)	13 (29.5)	15 (29.5)	2 (15.4)
IVS-I-6 (T>C)	26 (15.6)	6 (14.6)	1 (6.7)	0 (0.0)	10 (22.7)	7 (17.1)	2 (15.4)
−29 (A>G)	9 (5.4)	3 (7.3)	0 (0.0)	0 (0.0	0 (0.0)	6 (14.6)	0 (0.0)
polyA (T>C)	2 (1.2)	1 (2.4)	1 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
−88 (C>T)	2 (1.2)	1 (2.4)	0 (0.0)	0 (0.0)	1 (2.3)	0 (0.0)	0 (0.0)
−88 (C>A)	1 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.3)	0 (0.0)	0 (0.0)
Codon 24 (T>A)	1 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.4)	0 (0.0)
IVS-II-844 (C>A); IVS-II-839 (T>C)	1 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.4)	0 (0.0)
Unknown	1 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.3)	0 (0.0)	0 (0.0)
Hb S/β^{0}-thal	84 (50.3)	24 (58.5)	11 (73.3)	69.2)	15 (34.1)	18 (43.9)	7 (53.8)
IVS-I-1 (G>A)	21 (23.6)	11 (26.8)	0 (0.0)	0 (0.0)	4 (9.1)	5 (12.2)	1 (7.7)
IVS-I-2 (T>C)	1 (0.6)	0 (0.0)	1 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
IVS-I-2 (T>G)	1 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.3)	0 (0.0)	0 (0.0)
IVS-II-1 (G>A)	3 (1.8)	2 (4.9)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.3)	0 (0.0)
IVS-II-849 (A>G)	5 (3.0)	1 (2.4)	2 (13.3)	0 (0.0)	2 (4.5)	0 (0.0)	0 (0.0)
Codon 39 (C>T)	46 (27.5)	9 (22.0)	7 (46.7)	9 (69.2)	6 (69.2)	10 (24.4)	5 (38.5)
Codon 6 (−A)	2 (1.2)	1 (2.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.4)	0 (0.0)
Codons 106/107 (+G)	1 (0.6)	1 (2.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Unknown	4 (2.4)	0 (0.0)	1 (6.7)	0 (0.0)	1 (2.3)	1 (2.4)	1 (7.7)
TOTAL	167 (100.0)	41 (100.0)	15 (100.0)	13 (100.0)	44 (100.0)	41 (100.0)	13 (100.0)

HBH: Hhemocenter of Belo Horizonte; JFO: Hemocenter of Juiz de Fora; MOC: Hemocenter of Montes Claros; Hemope: Hemocenter of Prenambuco; Hemorio: Hemocenter of Rio de Janeiro; ITACI: Child Institute at Hospital das Clinicas da Faculdade de Medicina da Universidade de São Paulo.

IVS-I-110 (G>A): *HBB*: c.; IVS-I-5 (G>C): *HBB*: c.; IVS-I-5 (G>A): *HBB*: c.; IVS-I-6 (T>C): *HBB*: c.; −29 (A>G): *HBB*: c.; polyA (T>C): *HBB*: c.*110T>C; −88 (C>T): *HBB*: c.−138C>T; −88 (C>A): *HBB*: c.−138C>A; codon 24 (T>A): *HBB*: c.75T>A; IVS-II-844 (C>A): *HBB*: c.31-6C>A; IVS-II-839 (T>C): *HBB*: c.316-12T>C; IVS-I-1 (G>A): *HBB*: c.92+1G>A; IVS-1-2 (T>C): *HBB*: c.92+2T>C; IVS-I-2 (T>G): *HBB*: c.92+2T>G; IVS-II-1 (G>A): *HBB*: c.315-12G>A; IVS-II-849 (A>G): *HBB*: c.316-2A>G; codon 39 (C>T): *HBB*: c.118C>T; codon 6 (−A): *HBB*: c.20delA.

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Table 2.

Baseline laboratory data of individuals with Hb S/β-thalassemia according to the molecular mutation classification.

Parameters	Hb S/β^+-Thal (n=43)	Hb S/β^+-Thal (severe) (n=40)	Hb S/β^0-Thal (n=84)	p Value[a]
Hb (g/dL)[b,c]	10.6±2.0[e,f]	8.5±1.4[e]	8.5±1.5[f]	<0.001
WBC (10^9/L)[b,e]	7.3±2.9[e,f]	10.0±3.6[e]	11.0±3.8[f]	<0.001
Platelets (10^9/L)[b,c]	182.0±180.0[e,f]	343.5±232.0[e]	394.0±192.0[f]	<0.001
LDH (IU/L)[b,c]	234.0±78.0[e,f]	371.5±291.3[e,g]	464.0±252.5[f,g]	<0.001
Reticulocytes (%)[b,c]	1.9±2.1[e,f]	5.9±4.8[e]	7.0±5.9[f]	<0.001
Total bilirubin (μmol/L)[b,c]	0.8±0.3[e,f]	1.6±0.7[e]	1.8±1.5[f]	<0.001
Direct bilirubin (mg/dL)[b,c]	0.2±0.2[e,f]	0.5±0.2[e]	1.8±1.5[f]	<0.001
Hb S (%)[b,c]	65.5±5.8[e,f]	78.7±13.2[e]	80.5±1.4[f]	<0.001
Hb A (%)[b,c]	24.9±5.9[e,f]	4.7±11.9[e,g]	0.0±0.0[f,g]	<0.001
Hb F (%)[b,c]	3.7±7.1[e,f]	11.6±12.6[e]	13.0±18.7[f]	<0.001
Hb A_2 (%)[b,d]	5.2±1.4	5.8±1.3	5.7±1.8	0.069

Hb: hemoglobin; IQR: interquartile ranges; WBC: white blood cells count; LDH: lactate dehydrogenase.

[a] p Values refer to the Kruskal-Wallis test (for data presented as median ± IQR) and post hoc multiple comparisons by the Dunn test, or ANOVA (for data presented as mean ± SD), and post hoc multiple comparisons by the Tukey test.

[b] Variables with missing values.

[c] Data shown as median ± IQR.

[d] Data shown as median ± SD.

[e,f,g] Indicates pairs with significant difference in multiple comparisons.

Table 3.

Clinical complications and treatment data of individuals with Hb S/β-thalassemia according to the molecular mutation classification.

Parameters	**Hb S/β$^+$**	**Hb S/β$^+$-severe (*n*=40) (%)**	**Hb S/β^0 (*n*=84) (%)**	**Hb S/β$^+$ *vs.* Hb S/β$^+$-severe**	***p* Value[a]**	**Hb S/β$^+$ *vs.* Hb S/β^0**	***p* Value[b]**	**Hb S/β$^+$-severe vs. Hb S/β^0**	***p* Value[c]**
Clinical Complications	(*n*=43) (%)	(*n*=40) (%)	(*n*=84) (%)	OR (95% CI)		OR (95% CI)		OR (95% CI)	
Hospitalizations in the past 12 months	8 (18.6)	17 (42.5)	39 (46.4)	4.68 (1.63; 14.94)	**0.006**	0.23 (0.09; 0.54)	**0.002**	0.82 (0.36; 1.84)	0.632
VOE[d]	30 (69.8)	38 (95.0)	80 (95.2)	8.49 (2.01; 59.18)	**0.009**	0.11 (0.03; 0.36)	**<0.001**	1.15 (0.20; 9.07)	0.878
ACS[d]	18 (43.9)	28 (70.0)	52 (64.2)	3.54 (1.37; 9.78)	**0.011**	0.41 (0.18; 0.88)	**0.024**	1.28 (0.57; 2.98)	0.553
Splenic sequestration[d]	1 (2.3)	10 (25.6)	27 (32.9)	15.80 (2.71; 302.53)	**0.011**	0.04 (0.002; 0.21)	**0.002**	0.68 (0.27; 1.63)	0.399
Ischemic stroke[d]	–	–	3 (3.7)	–	–	–	–	–	–
TCD performed[d]	17 (40.5)	20 (52.6)	43 (53.1)	2.24 (0.77; (6.93)	0.147	0.44 (0.18; 1.03)	0.064	0.80 (0.32; 2.04)	0.643
Abnormal TCD[d]	1 (5.9)	6 (30.0)	3 (7.0)	8.05 (1.12; 166.18)	0.073	0.95 (0.04; 10.33)	0.967	5.86 (1.29; 33.36)	**0.028**
Leg ulcers[d]	1 (2.3)	1 (2.6)	5 (6.0)	0.85 (0.03; 24.43)	0.914	0.34 (0.02; 3.11)	0.470	0.42 (0.02; 3.02)	0.451
Avascular necrosis	3 (7.0)	5 (12.5)	10 (11.9)	2.30 (0.47; 13.20)	0.314	0.66 (0.13; 2.64)	0.575	1.25 (0.33; 4.33)	0.733
Sepsis/bacteremia	2 (4.7)	4 (10.0)	2 (2.4)	3.08 (0.54; 24.30)	0.224	1.57 (0.18; 14.00)	0.664	5.87 (1.05; 445.37)	0.052
Acute renal failure	0 (0.0)	1 (2.5)	1 (1.2)	–	–	–	–	–	–
Chronic renal failure	0 (0.0)	0 (0.0)	0 (0.0)	–	–	–	–	–	–
Priapism[d]	2 (7.4)	2 (11.1)	7 (15.9)	1.4 (0.14; 14.33)	0.762	0.46 (0.06; 2.57)	0.406	0.62 (0.07; 3.73)	0.623
Pyelonephritis	0 (0.0)	1 (2.5)	0 (0.0)	–	–	–	–	–	–
Treatment Data	*n* (%)	*n* (%)	*n* (%)						
Splenectomy	0 (0.0)	12 (30.0)	29 (34.5)	–	–	–	–	0.85 (0.36; 1.94)	0.709
Cholecystectomy	1 (2.3)	6 (15.0)	17 (20.2)	7.09 (1.10; 139.20)	0.079	0.09 (0.005; 0.46)	**0.021**	0.78 (0.25; 2.13)	0.635
HU therapy	4 (9.3)	14 (35.0)	39 (46.4)	5.81 (1.78; 23.21)	**0.006**	0.12 (0.03; 0.33)	**<0.001**	0.65 (0.29; 1.41)	0.282
Blood transfusions in the past 12 months	2 (4.7)	9 (22.5)	18 (21.4)	8.01 (177; 58.41)	**0.015**	0.16 (0.02; 0.61)	**0.020**	1.11 (0.43; 2.75)	0.821
Ever transfused	15 (34.9)	32 (80.0)	74 (88.1)	7.01 (2.66; 20.22)	**<0.001**	0.07 (0.02; 0.17)	**<0.001**	0.60 (0.21; 1.74)	0.334
Chronic transfusions	0 (0.0)	0 (0.0)	1 (1.1)	–	–	–	–	–	–

OR (95% CI): odds ratio (95% confidence interval); VOE: vaso-occlusive event; ACS: acute chest syndrome; TCD: transcranial Doppler.

[a] *p* Value for Hb S/β$^+$ *vs.* Hb S/β$^+$-severe.

[b] *p* Value for Hb S/β^+ *vs.* Hb S/β^0.

[c] *p* Value for Hb S/β^+-severe *vs.* Hb S/β^0.

[d] Variables with missing values.