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ORIGINAL ARTICLE

Molecular Characterization and Phenotypical Study of β-Thalassemia in Tucumán, Argentina

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Abstract

The main hereditary hemoglobin (Hb) disorder in Argentina is β-thalassemia (β-thal). Molecular studies performed in the center of the country exhibited a marked prevalence of the codon 39 (C > T) and IVS-I-110 (G > A) mutations. The northwest region of Argentina has a different demographic history characterized by an important Spanish influx. Seventy-one β-thal carriers attending the Instituto de Bioquímica Aplicada, Tucumán, Argentina, were investigated for β-globin gene mutations by real-time polymerase chain reaction (RT-PCR). To examine the genotype-phenotype relationship, mean corpuscular volume (MCV), mean corpuscular Hb (MCH) and Hb A_2 were measured. In order to recognize β-thal, Mentzer Index, Shine & Lal and Red Cell Distribution Width Index (RDWI), were calculated. The ethnic background of subjects revealed that 82.0% of the population was of Italian, Spanish and Arab origin. Seven mutations were detected: codon 39 (45.0%), IVS-I-1 (G > A) (22.5%), IVS-I-110 (16.3%), IVS-II-1 (G > A) (4.1%), IVS-I-1 (G > T) (2.0%), IVS-I-6 (T > C) (2.0%) and IVS-II-745 (G > C) (2.0%). In three families (6.1%), β-thal mutations were not determined. These results differed from other Argentinian studies because at present codon 39 and IVS-I-1 are the most prevalent; MCV, MCH and Hb A_2 did not correlate with the type of mutation (β^0/β^+). Values of MCV (67.0 fL) and Hb A_2 (4.85%) were unable to discriminate between them. Significant differences ($p < 0.05$) in MCV, MCH and Shine & Lal were observed between the undetermined group and the three most common mutations. These data show different patterns of β-thal mutations in the center and northwest regions of Argentina. Differences might represent the influence of Spanish immigration.

Keywords

Argentina, β-thalassemia (β-thal) mutations, genotype-phenotype correlation

History

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Introduction

Thalassemias are caused by genetic defects affecting the globin genes encoding for the α and β chains of the hemoglobin (Hb) molecule. Some of the mutations reduce or eliminate the *HBB* gene expression, leading to Hb deficiency and β-thalassemia (β-thal) (1). Almost 300 β-thal alleles have now been characterized (2). The spectrum of mutations differs in ethnic groups of each population and includes a few common mutations and a variable number of rare ones (3).

Mutations that completely inactivate the β gene resulting in no β-globin production cause β^0-thal. Other mutations allow the production of some β-globin and, depending on the degree of quantitative reduction in β chains output, are classified as β^+- or β^{++}- (silent) thal. A quantitative reduction in β-globin results in the accumulation of excess α-globin chains that are responsible for the pathophysiology of the disorder (4). Thus, the severity of the phenotype is usually related to the degree of imbalance between α- and non α-globin chain synthesis and to the size of the free α chain pool (5). The excess of α-globin chains aggregate in red cell precursors forming inclusion bodies, causing mechanical damage and their premature destruction in the bone marrow, a process named ineffective erythropoiesis. Surviving red cells that reach peripheral circulation are prematurely destroyed. Thus, anemia in β-thal results in a combination of ineffective erythropoiesis, peripheral hemolysis and an overall reduction in Hb synthesis. Therefore, factors which reduce the degree of chain imbalance and the magnitude of α chain excess in red cell precursors will have an ameliorating effect on the phenotype. At the primary level, this is directly related to the severity of the β-thal mutation itself; at the secondary level, to the effects of genetic variability at two other loci, the α and γ gene loci (6). Several investigators have described significant differences between β^0- and β^+-thal in hematological parameters such as mean corpuscular volume (MCV), mean corpuscular Hb (MCH) and Hb A_2 levels (7–9).

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Thalassemias occur at high frequencies in tropical and subtropical regions, where malaria is or has been endemic. It has a high incidence in a broad band extending from the Mediterranean Basin and parts of Africa throughout the Middle East, the Indian sub-continent, Southeast Asia, Melanesia and into the Pacific Islands. Carrier frequency for β-thal in these areas ranges from 1.0 to 20.0%, rarely greater. As a result of population migration and the African slave trade, these diseases have spread throughout the world and are now an important health problem in the Americas (10). The Spanish colonization was mainly responsible for the introduction of β-thalassemic genes in Argentina, and its influence still visible today. Subsequent migration waves from the Mediterranean Basin, particularly from Italy and Spain during the 19th and 20th centuries, also contributed greatly. The number of immigrants quickly equaled the number of natives and a mixed population resulted. Previous hematological studies showed a 0.8% incidence of thalassemic carriers among 4000 blood donors living in the Greater Buenos Aires area (11). Larregina *et al.* (12) studied 8738 patients in Bahía Blanca, Argentina, and found β-thal trait in 81 patients (0.9%). In Tucumán, β-thal trait is the most commonly inherited anemia, with no records on prevalent mutations in the region (13).

The spectrum of mutations in Argentina has been studied by several research groups of Buenos Aires, Santa Fe and Córdoba provinces (14–17). The four most frequent mutations presented in their studies were codon 39 (C > T), IVS-I-110 (G > A), IVS-I-1 (G > A) and IVS-I-6 (T > C). The purpose of this study was to define the profile of β-thalassemic mutations in the province of Tucumán and to compare them with those reported for other regions of Argentina. Furthermore, it aimed at examining the hematological phenotype and its potential relationship with the genotype in β-thal carriers.

Materials and methods

A descriptive cross-sectional study was carried out at the Instituto de Bioquímica Aplicada, Universidad Nacional de Tucumán, Tucumán, Argentina from September 2011 to December 2013. β-thalassemia carriers from Tucumán and adjoining regions comprising Santiago del Estero and Salta provinces (Argentina) were included.

Subjects

This study was approved by the Provincial Health System Ethical Committee, Tucumán, Argentina. The study sample consisted of 71 β-thal carriers belonging to 49 families. Family history of hemoglobinopathies and ethnic background of the patients were recorded.

Hematological studies

Blood samples of β-thal carriers were collected in EDTA-containing vacutainers. Red blood cell (RBC) count, MCV, MCH and MCHC (mean corpuscular Hb concentration) were analyzed with a Sysmex KX-21N (Kobe, Japan) hematological counter. Furthermore, discrimination RBC indices such as Mentzer Index (MCV/RBC), Shine & Lal ($MCV^2 \times MCH \times 0.01$) and RDWI (Red Blood Cell Distribution Width Index = MCV × RDW/RBC) were calculated. Decreased MCV and MCH allowed considering an individual as a potential carrier. The carrier status was confirmed by cellulose acetate electrophoresis (CAE) at alkaline pH, and Hb A_2 level by microcolumn chromatography (BioSystem S.A., Barcelona, Spain). If the Hb A_2 level was ≥3.5%, then the subjects were diagnosed as β-thal carriers. All patients with microcytic hypochromic anemia were included for mutational analysis. Hb F was quantified by an alkali denaturation technique only when an increased level was detected in CAE. Serum iron was measured by a colorimetric method (Wiener Lab, Rosario, Argentina).

DNA analysis

Genomic DNA was isolated with the High Pure polymerase chain reaction (PCR) Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany) from 250 μL of EDTA-anticoagulated whole blood. Polymerase chain reaction and mutation detection by melting curve analysis were performed on the LightCycler 2.0 (Roche Diagnostics GmbH), which can simultaneously measure signals emitted from two different fluorophores. The PCR primers (forward: 5′-GCT GTC ATC ACT TAG ACC TCA-3′; reverse: 5′-CAC AGT GCA GCT CAC TCA G-3′) were designed to amplify a 587 bp region of the β-globin gene surrounding the most common β-thal mutations in the Argentinian population (and most of the common mutations in all world populations), as described by Vrettou *et al.* (18). The clustering of many mutations within small distances permitted the use of two combinations (or sets) of probes to analyze five common β-thal mutations. The acceptor and donor probes in sets A and B were labeled with different fluorophores.

Set A

Mutations: IVS-I-110 and codon 39; hybridization probes: acceptor, IVS-I-110 (5′-TCT GCC TAT TGG TCT ATT TTC CC-3′-LC Red 640), melting temperature (Tm) 56.6 °C; acceptor codon 39 (TYE 705-5′-ACC CTT GGA CCC AGA GGT TCT T-3′-phosphate) Tm 56.0 °C; donor IVS-I-110/codon 39 (FITC-5′-CCC TTA GGC TGC TGG TGG TC-3′-FITC), Tm 61.6 °C.

Set B

Mutations: IVS-I-1, IVS-I-5 (G > A) and IVS-I-6 (T > C); hybridization probes: acceptor, IVS-I-1.5.6 (TYE 705-5′-TGT AAC CTT GAT ACC AAC CTG CCC A-3′-phosphate), Tm 63.2 °C; donor: IVS-I-1.5.6 (5′-TGC CCA GTT TCT ATT GGT CTC CTT AAA CCT GTC-3′-FITC), Tm 68.4 °C. The IVS-I-1 mutation was confirmed by genomic DNA sequencing.

The PCR amplification reactions were carried out in LightCycler glass capillary tubes (Roche Diagnostics) in a total reaction volume of 20 μL containing the ready-to-use reaction mixture provided by the manufacturer (LightCycler DNA Master Hybridization Probes; Roche Diagnostics GmbH) with 4 mM MgCl, 0.5 μL of each PCR primer, and 0.15 μL each fluorescent probe. The PCR amplification included a first denaturation step of 30 seconds at 95 °C followed by 35 cycles at 95 °C for 3 seconds, 58 °C for 5 seconds, and 72 °C for 20 seconds with a temperature ramp

of 20 °C/seconds. During PCR, emitted fluorescence was measured at the end of the annealing step of each amplification cycle to monitor amplification.

Genotypes were determined by melting curve analysis of probes immediately after the amplification step. This involved a momentary increase in temperature up to 95 °C, cooling to 45 °C for 2 min. to achieve maximum probe hybridization, and then heating to 85 °C at a rate of 0.4 °C/seconds, during which time the melting curve was recorded. Mutations that were not detected with the probe sets employed were submitted to genomic DNA sequencing (Applied Biosystems 3130 Genetic Analyzer/Hitachi Ltd.; Foster City, CA, USA).

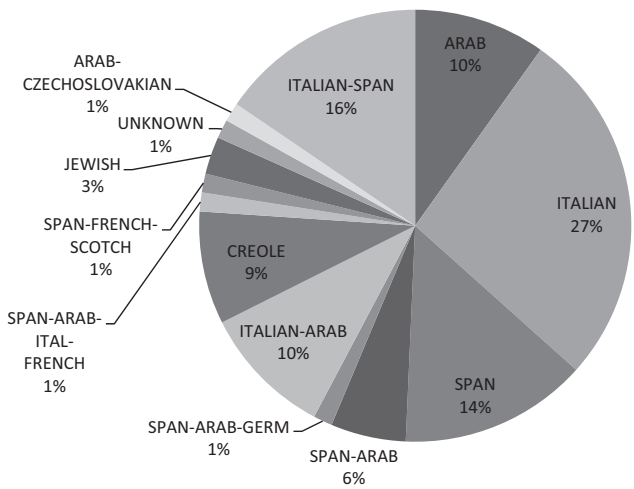
Statistical analysis

The Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) software version 17.0 and Microsoft Office Excel version 2010, were used throughout to carry out all the statistical analyses. Hematological parameters were compared by analysis of variance (ANOVA) followed by the Student's *t*-test for pairwise comparisons between groups. The tests were done with $p < 0.05$ as the level of significance.

Results

Forty-nine families with β-thal from Northwest Argentina were studied. Seventy-one β-thal carriers (46 unrelated chromosomes) were detected; 53 were adults (31 females and 22 males) aged 13–76 years, and 18 were children (12 females and 6 males) aged 1–12 years. These individuals represent a number of different ethnic groups: 19 Italians, 11 Italian-Spaniards, 10 Spaniards, seven Arabs, seven Italian-Arabs, six Creoles, four Spanish-Arabs, two Jews and five others, who included individuals descended from parents of other European countries (France, Germany, Czechoslovakia and Scotland). Eighty-two percent of the sample was Italian, Spanish and/or Arab (Figure 1).

Fifty-two individuals were carriers of β^0 mutations (73.2%), 13 of β^+ mutations (18.3%) and six (8.5%) of unknown mutations (ND group). The β-thal mutations detected in frequency order were codon 39, IVS-I-1 (G > A), IVS-I-110, IVS-II-1 (G > A), IVS-I-1 (G > T), IVS-I-6 and IVS-II-745 (C > G) (Table 1). In the three most prevalent mutations, the main countries of origin of β-thal carriers were Italy for codon 39 and IVS-I-110, and Spain for IVS-I-1 (G > A) (Table 2).



ABBREVIATIONS: SPAN, Spaniards; ITAL, Italians; GERM, Germans

Figure 1. Ethnic background of β-thal carriers.

Hematological parameters according to mutation are shown in Table 3. Significant differences ($p < 0.05$) were observed in MCV, MCH and Shine & Lal between the ND group and the codon 39, IVS-I-1 and IVS-I-110 groups. No significant differences ($p > 0.05$) were found between the β^0 and β^+ group in the hematological parameters. Figures 2 and 3 are scattergrams of the MCV and MCH of the subjects, respectively, grouped according to the mutations they carry. There was a wide range of MCV values (57.0 to 80.0 fL) and their distribution (Figure 2) is non random. For all recognized mutations there was an overlap in the MCV ranges, but four individuals in the ND and IVS-I-1 groups showed higher MCV than the rest. The MCH data of these individuals and their mutations were also evaluated (Figure 3). Overall, the visual spread of the points was found to be similar to the MCV data. An MCV value of 67.0 fL recommended by Rund *et al.* (8) was used to discriminate β^0- and β^+-thal. If the MCV was higher than 67.0 fL, the mutation was β^+, and if the MCV was lower, it was β^0. This process allowed the detection of 89.0% (46/52) of β^0 and 23.0% (3/13) of β^+ mutations.

Table 1. Mutations found in β-thalassemia carriers in Tucumán, Argentina.

Mutation	Type[a]	Distribution[a]	Families	Patients	Alleles
Codon 39 (C > T)	β^0	Mediterranean	22	31	22
IVS-I-110 (G > A)	β^+	Mediterranean	8	10	8
IVS-I-1 (G > A)	β^0	Mediterranean; Middle East	11	15	11
IVS-I-1 (G > T)	β^0	Asian Indian; Southeast Asian; Chinese	1[b]	2	1
IVS-II-1 (G > A)	β^0	Mediterranean; US Blacks	2	4	2
IVS-I-6 (T > C)	β^{++}	Mediterranean	1	2	1
IVS-II-745 (C > G)	β^+	Mediterranean	1	1	1
Unknown	–	–	3	6	–
Total	–	–	49	71	46

[a]References to these mutations can be found in (13).
[b]The family has Italian and Syrian ancestors.

Table 2. Country of origin of the carriers with the most prevalent β-thalassemia mutations.

Origin	Codon 39 (C > T)	IVS-I-1 (G > A)	IVS-I-110 (G > A)
Italy	9	1	4
Spain	6	6	2
Arab countries	4	3	0
Spain; Arab countries; Germany	0	1	0
Spain; Arab countries	1	1	1
Italy; Spain	5	2	3
Italy; Arab countries	2	1	0
Spain; Arab countries; Italy; France	1	0	0
Spain; France; Scotland	1	0	0
Arab countries; Czechoslovakia	1	0	0
Unknown	1	0	0
Total	31	15	10

Table 3. Hematological parameters in β-thalassemia carriers according to mutation.

Parameters	Codon 39 ($n=31$)	IVS-I-1 ($n=17$)	IVS-I-110 ($n=10$)	IVS-II-1 ($n=4$)	IVS-I-6 ($n=2$)	IVS-II-745[a] ($n=1$)	ND Group ($n=6$)
MCV (fL)	64.3 ± 2.9	64.3 ± 4.6	64.9 ± 1.9	63.4 ± 2.5	62.8 ± 0.6	67.4	71.1 ± 6.8[b]
MCH (pg)	19.1 ± 0.9	19.0 ± 1.5	19.3 ± 0.7	18.6 ± 0.7	18.4 ± 0.1	19.8	21.5 ± 2.7[b]
Hb A_2 (%)	5.1 ± 0.8	4.8 ± 0.6	5.1 ± 0.7	5.7 ± 1.0	5.2 ± 0.2	5.1	5.1 ± 0.6
RDW (%)	16.7 ± 1.9	16.6 ± 1.3	15.8 ± 1.3	18.0 ± 1.8	17.3 ± 0.8	15.6	15.3 ± 1.2
Mentzer Index	11.1 ± 1.2	11.6 ± 2.3	11.3 ± 1.1	10.6 ± 0.8	10.8 ± 0.6	12.9	13.0 ± 2.1
Shine & Lal	795.0 ± 102.0	797 ± 203.0	815.0 ± 75.0	749.0 ± 86.0	726.0 ± 9.0	899.0	1114.0 ± 348.0[b]
RDWI	186.0 ± 23[c]	192.0 ± 48.0	178.0 ± 16.0	189.0 ± 7.0	186.0 ± 1.0	201.0	196.0 ± 26.0
Iron (μg/dL)	99.0 ± 50.0	101.0 ± 43.0	104.0 ± 37.0	99.0 ± 17.0	64.0 ± 2.0	81.0	91.0 ± 30.0

ND group: not determined group; MCV: mean corpuscular volume; MCH: mean corpuscular Hb; RDW: red cell distribution width; RDWI: Red Cell Distribution Width Index.
[a]The IVS-II-745 group was excluded from the comparative analysis.
[b]The p value between the ND group and the codon 39, IVS-I-1 and IVS-I-110 groups was of <0.05.
[c]In one case, the RDWI value was not available.

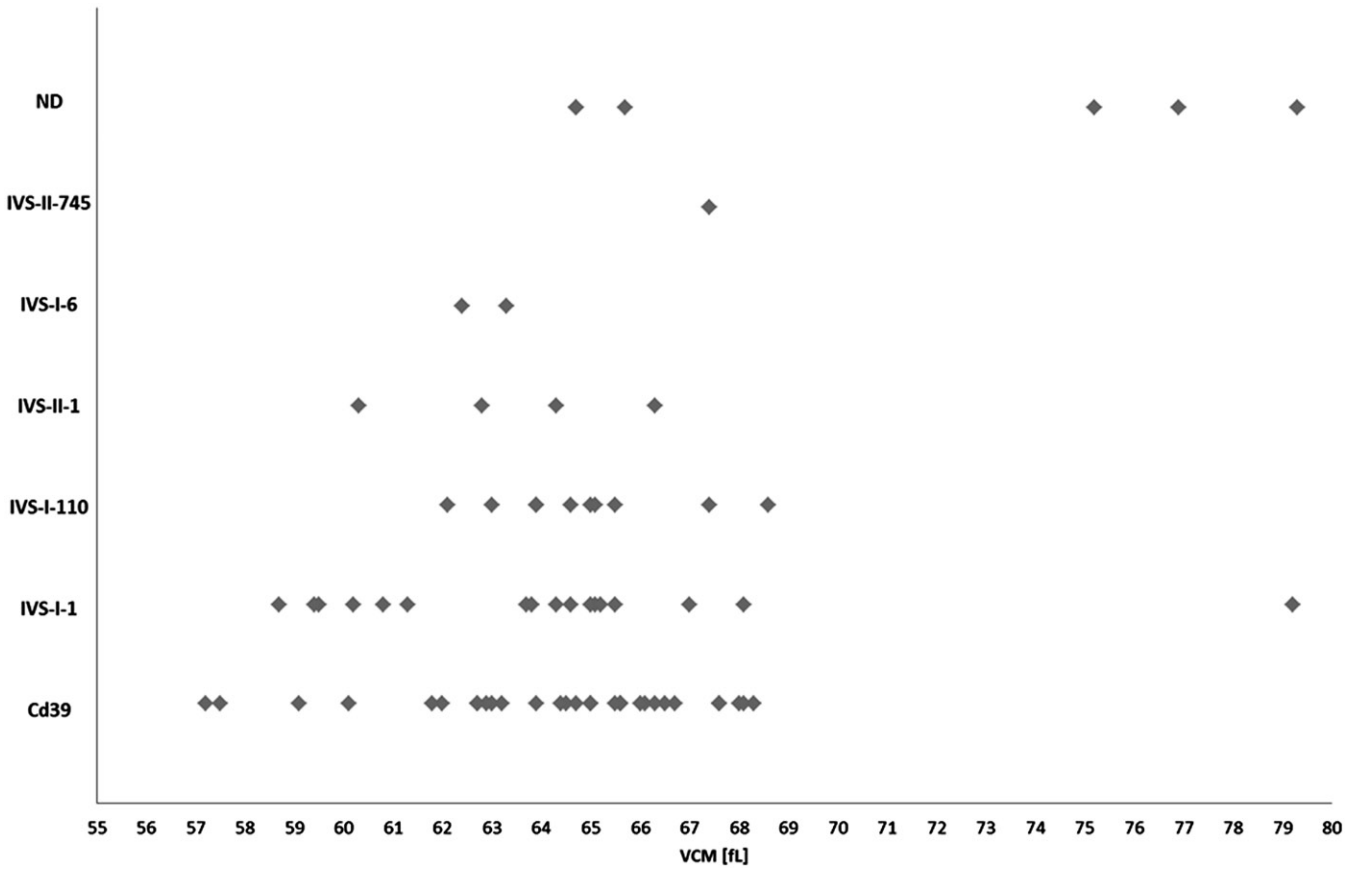


Figure 2. Relationship between MCV and mutation in heterozygotes for β-thal.

Although the sensitivity of the method was 88.0%, specificity reached only 23.0%.

Hb A_2 levels exhibited a considerable overlap in values. The analysis showed no significant differences between the β^0 and β^+ groups ($p=0.837$), which meant that Hb A_2 could not be used to predict the type of β mutation. However, the Hb A_2 value of 4.85%, recommended by López-Escribano *et al*. (19), was used to discriminate between thalassemia phenotypes. If Hb A_2 was higher than 4.85%, the mutation was β^0, and if Hb A_2 was lower, it was a β^+ mutation. The procedure allowed the determination of 60.0% (31/52) β^0 and 23.0% (3/13) β^+ mutations. Method sensitivity was 60.0% and specificity 23.0%. Serum iron was used to recognize iron deficiency in two patients with β^0 mutations and Hb A_2 levels of <4.85%. All patients showed normal Hb F levels, except a 2-year-old child.

The discrimination indices Mentzer Index, Shine & Lal and RDWI permitted the identification of the β-thal trait in 93.0% (66/71), 99.0% (70/71) and 94.0% (66/70) of the cases, respectively. Using the three indices together allowed the detection of 100.0% of β-thalassemic individuals, since at least one was positive.

Table 4 compares the current results with other studies published by Argentine researchers (14–17). The comparative

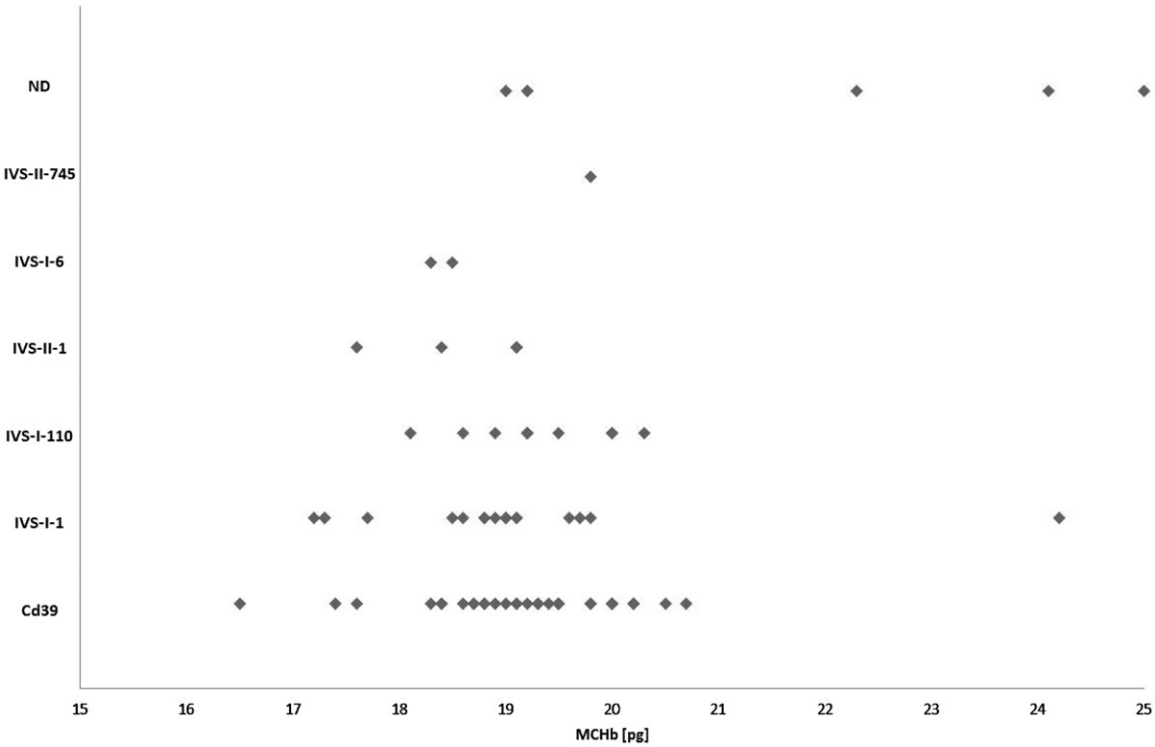


Figure 3. Relationship between MCH and the type of β mutation.

Table 4. Frequency distribution of seven β-thalassemia alleles in the Argentinean population.

Mutations	This Study (%)	Rosario; Santa Fe (%)	Buenos Aires; Other Regions (%)	Buenos Aires (%)	Córdoba (%)
Codon 39 (C > T)	45.0	57.4	45.7	47.0	29.2
IVS-I-110 (G > A)	16.3	22.1	23.2	22.3	58.3
IVS-I-1 (G > A)	22.5	4.9	10.7	9.4	3.8
IVS-I-1 (G > T)	2.0	ND	ND	ND	ND
IVS-I-6 (T > C)	2.0	2.5	7.9	5.9	ND
IVS-II-1 (G > A)	4.1	1.6	2.5	3.5	ND
IVS-II-745 (C > G)	2.0	4.1	0.7	2.3	ND
Unknown	6.1	7.4	4.3	4.7	0.0
Others	0.0	ND	5.0	4.7	8.3
References		13	14	15	16

ND: not determined.

study revealed differences, because all of them reported that the most common β-thalassemic mutations are codon 39 and IVS-I-110, while the current study found that the most frequent mutations in Tucumán are codon 39 and IVS-I-1 (G > A).

Discussion

There are more than 300 different known β-thal mutations worldwide, 12 of which have also been reported for Argentina (15). In this study population, seven different mutations were detected, the most frequent being codon 39 (45.0%), IVS-I-1 (G > A) (22.5%) and IVS-I-110 (16.3%). Molecular studies performed in the center region of the country (14–17) showed a marked prevalence of the codon 39 (29.2–57.4%) and IVS-I-110 (22.1–58.3%) mutations, with a minor contribution of IVS-I-1 (G > A) (3.8–10.7%). However, the northwest region of the country has a different demographic history, characterized by the absence of a massive Italian immigration and an important Spanish and Arab arrival.

The ethnic background of the subjects studied revealed that 82.0% of the population had an Italian, Spanish and/or Arab origin. The three unknown chromosomes belonged to Jewish, Italian and Spanish-Arab families. In the case of a Jewish family, one of the reasons for it not being detected in the analysis could be the different racial origin.

In Argentina, the first flow of migrants from Spain, at the beginning of the 16th century, created urban settlements. Thus, initially, the Argentine population grew on the basis of Spaniards, their descendants (Creoles), aborigines, Africans (slaves) and mixed races (16). Between 1850 and 1955, subsequent migration waves, mainly from Italy and Spain, also contributed greatly. The provinces of Buenos Aires, Santa Fe, Entre Ríos, La Pampa and Córdoba received the highest number of European immigrants, while in the region of Cuyo, only Mendoza excelled at receiving immigrants. In the northwest, Tucumán received the highest migration flow due to the expansion of the sugar industry (20). Between 7000 and 10,000 Italian immigrants arrived in Tucumán between the First and the Second World Wars; and today,

there are 4370 Italian citizens residing in Tucumán, between natives and descendants (21). Arab immigration in Argentina is the third largest in the country after the Spanish and Italians. The strongest community is Syrian-Lebanese, followed by the Iraqi and Palestinians. In the 1908–1914 period, Tucumán received a massive influx of immigrants from the Ottoman Empire (the Balkan peninsula south of the Danube river, across Anatolia and the Arab world from Iraq to North Africa); 1210 people migrated officially and around 2950 did so spontaneously (22). According to the second census of the Argentine Republic, 2.0% Spanish, 0.6% Italian and 2.4% Arab immigrants lived in Tucumán in 1895 (23). These facts could account for the ethnic composition found in β-thal carriers, which is slightly different from that reported by other researchers. They reported that 65.1–89.3% of patients originated from Italy, 9.8–25.4% from Spain and 0.0–6.0% from Arab countries (14–16).

It is known that it would be useful to differentiate with hematological parameters between β^0- and β^+-thal in heterozygous carriers. However, when β^0- and β^+-thal groups were compared, no differences were observed. Attempts to do this have been made in various populations such as Jews, Spaniards, Argentinians, Italians and Greeks (7–9,14,24). Bragós *et al.* (14) did not find significant differences ($p > 0.05$) in red cell indices and Hb A_2 as in the present study, and Stefani *et al*. (24) did not observe modifications in MCV and MCH when comparing β^0- and β^+-thal. Rund *et al*. (8) and López-Escribano (9) detected significant changes in MCV, MCH and Hb A_2 values between the same groups. Heterozygotes for IVS-I-6 and −87 (C > G) mutations had higher MCV and MCH compared to heterozygotes for other β-thal mutations such as codon 39, IVS-I-110, IVS-I-1 (G > A), and IVS-II-745 (7). In Israel, Rund *et al*. (8) communicated that, in almost all cases, carriers of β^0 mutations had an MCV value below 67.0 fL, whereas all but a few β^+ heterozygotes had MCV levels above this cut-off point. However, β-thal individuals did not behave in the same way; although the recommended value was able to identify 89.0% of β^0-thal carriers, the specificity of the method reached only 23.0%. Hidden iron deficiency could be responsible for the difference between research groups. Although serum iron was measured, the best parameter to detect iron deficiency is ferritin.

Two European groups reported significantly lower levels of Hb A_2 in IVS-I-6 β-thal carriers when matched with those who shared the IVS-I-110 mutation (7,24). The small number of IVS-I-6 patients in this study would probably account for the difference. The Hb A_2 level was not a useful parameter to discriminate between both types of carriers (β^0 or β^+), perhaps due to the technique used to quantify Hb A_2. Rund *et al*. (8) determined Hb A_2 spectrophotometrically after electrophoretic separation on cellulose acetate; Stefanis *et al.* (24) measured it using HPLC; Rosatelli *et al*. (7) quantified it by microchromatography, and the other authors did not specify the method (7–9,14,24). Hb A_2 was measured by microcolumn chromatography and the manufacturer informed that no significant differences were observed with HPLC.

In an Indian population of heterozygous and homozygous β-thal individuals, a correlation of genotype with phenotype expressed in terms of clinical severity, *i.e.* mild, moderate and severe, was perceived (25). Such comparison could not be performed in this study because the sample was only composed of heterozygous β-thal patients with mild anemia and microcytosis.

Significant modifications in MCV, MCH and Shine & Lal were detected between the ND group and the codon 39, IVS-I-1 (G > A) and IVS-I-110 groups, the ND group showing higher MCV, MCH and Shine & Lal values. Some of the unidentified mutations would likely be β^+ type or could present coinheritance of βα-thal, which is currently under study.

Investigators have long sought a reliable mathematical index based on routine complete blood count results to help differentiate between microcytosis due to iron deficiency and that due to a thalassemia syndrome. Previous studies conducted in this laboratory established that the use of the Mentzer Index, Shine & Lal and RDWI was more reliable than any other published index (26) to identify and discriminate β-thal from iron deficiency anemia. The use of the three indices in conjunction helped to identify 100.0% of the patients. No index has yet been found to offer both 100.0% sensitivity and specificity, and a different index for each population has been recommended. In Turkey, Shine & Lal and Green & King index offered the best discrimination and RDWI, the worst (27). In Greece, Green & King showed the highest reliability (28). To increase the effectiveness of screening, a combination of tests has been used by a number of laboratories. Manglani *et al.* (29) incidentally found that the naked eye single tube red cell osmotic fragility test (NESTROFT) in combination with MCV <80.0 fL, did not miss identification of even a single case of thalassemia trait, thereby achieving a sensitivity of 100.0%. Sahli *et al.* (30) correctly identified 76.0–80.0% of children with β-thal trait and iron deficiency anemia using Srivastava Index (MCH/RBC), MCH, RBC, Mentzer Index or MCHC. However, a combination of Mentzer Index and Srivastava Index (at least one of them was positive) raised the percentage to 88.0% (30).

Two molecular alterations have been identified as the most common in the Mediterranean area: an RNA processing mutation IVS-I-110 and a nonsense mutation at codon 39. IVS-I-110 was most prevalent in countries on the eastern shore of the Mediterranean, where Turkey (35.3%) (31), Lebanon (34.2%) (32) and Cyprus (74.0–80.0%) (33) displayed the highest frequencies. Codon 39 occurred in 40.0% of β-thal from the western part of the Mediterranean Basin (34). In the middle of the Mediterranean area, both mutations showed similar frequencies (35). In Spain, molecular studies of β-thal made in different regions reflected that the most common anomalies were the codon 39 mutation, which presented between 20.0 and 64.0% of β-thal, and IVS-I-1 (G > A), which in some regions reached 60.0% (36). However, this situation is changing, as demonstrated by Pereira *et al.* (37) since although codon 39 is still the most prevalent mutation in Catalonia, the relative percentage has decreased from 64.0 to only 27.85%, while IVS-I-1 (G>A) has increased from 3.5 to 16.45%. Therefore, the present results might represent the influence of migrations from Spain to the northwest region of Argentina. This fact was corroborated by examining the country of origin of the IVS-I-1 (G > A) carriers.

The second major mutation found in Indo-Asians (38–40), *i.e.* IVS-I-1 (G>T) was detected for the first time in Argentina in an Italian-Arab family. It is a rare observation in contrast to other parts of Argentina and other South American countries such as Venezuela and Brazil (41–43).

Finally, it can be concluded that the genotypic profile of β-thal shows variability in the Argentinian population. Hence, it would be arbitrary to infer regional study results as being representative of the whole Argentinian population. Argentinian researchers of different regions should identify their most frequent genotypes to provide a better understanding of this disease and design adequate public health policies. This study throws light on a genotype-phenotype correlation of β-thal mutations in Tucumán, Argentina. As elsewhere in the country, a relationship between genotype and hematological phenotype was not observed. The cause for this could be a hidden iron deficiency that needs to be considered.

Declaration of interest

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