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ORIGINAL ARTICLE

Characterization of deletional and non-deletional alpha globin variants in a large cohort from Spain between 2009 and 2014

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Abstract

The hemoglobinopathies are a group of disorders passed down through families (inherited) in which there is abnormal production or structure of the hemoglobin molecule. They are among the most common inherited diseases around the world. Those that produce abnormal hemoglobin are called structural hemoglobinopathies while thalassemia is another type of disorder that is caused by a defect in the gene production of the globin chains. In a study ambispective comprising 1623 patients, 153 subjects showed an abnormal hemoglobin and 1470 with hypochromic and microcytic anemia, and of these 1470, 23 patients were studied for simultaneously α-thalassemias and structural hemoglobinopathies. Among the α-thalassaemia cases, 1282 cases (87.2%) were deletional α-thalassemia, 172 cases (11.7%) were non-deletional α-thalassemia, and 16 cases (1.1%) were deletional and non-deletional α-thalassamias simultaneously. Thus, approximately 12% of the cases were non-deletional α-thalassaemia. Clinical diagnosis, only 19 severe cases (1 hydrops fetalis and 18 instances of Hb H disease), 1200 thalassamias traits, and 160 thalassaemia silent carriers were recorded within the α-thalassaemia. Regarding structural hemoglobinopathies, there were only 2 cases of hemoglobinopathies with low oxygen affinity and 1 case of hemoglobin M; the remaining 150 were silent hemoglobinopathies. Non-deletional α-thalassaemia represented 12% of all α-thalassemias in our region; the most common deletion in our area was the 3.7-kb deletions, followed by Asian –(SEA) and –(FIL). The alterations responsible for non-deletional α-thalassaemia are most represented by the Hph and Hb Groene Hart and, in the case of structural hemoglobinopathies, Hb Le Lamentin and Hb J-Paris.

Keywords Alpha thalassemia · Deletion and non-deletion · Structural hemoglobinopathies · Molecular diagnosis

Background

Thalassemias are a heterogeneous group of inherited anemias that are characterized by the reduction or total absence of the synthesis of one or more globin chains. These chains that are synthesized, to a lesser extent, are usually standard in composition and structure [1]. Thalassemias are microcytic and hypochromic anemias that have a specific hemolytic component [2].

Structural hemoglobinopathies are inherited disorders of hemoglobin synthesis, where the sequence of one of the globin chains, those which form hemoglobin, is altered [3].

More than 1000 hemoglobin variants have been described, of which approximately half do not result in pathology. In pathological cases, mutations may produce an abnormal polymerization of hemoglobin by altering the surface charge (Hb S); precipitation of hemoglobin in the red blood cell (unstable hemoglobins), changes in oxygen affinity (polycythaemia and cyanosis), or increased oxidation of the heme iron group (methemoglobins or Hb M). Also, hemoglobinopathies may alter their electrophoretic mobility, which both intentionally and causally contributes to their identification [4].

The main objectives of this work were to analyze the distribution of demographic variables such as sex, age, or ethnicity of patients with α-thalassemia and/or structural hemoglobinopathies in our country, to examine the prevalence of non-deletional α-thalassemia within all cases of α-thalassemia to discern whether it conforms to the results of previous studies in other populations and to determine the molecular heterogeneity of α-thalassemia and structural hemoglobinopathies in Spain.

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Material and methods

Our laboratory is a reference center in Spain for the study and molecular diagnosis of structural hemoglobinopathies and thalassemias, receiving approximately 850 samples annually. Samples (8250) were received from January 2009 to December 2014, of which 1623 individuals showed some alteration in the alpha globin genes. This manuscript is a study ambispective comprising these 1623 individuals with some modification in the alpha globin genes, and 153 subjects showed some abnormal hemoglobin and 1470 with hypochromic and microcytic anemia, of these 1470, 23 patients were studied for simultaneous α-thalassemias and structural hemoglobinopathies.

Studied subjects comprised both native (79.7%) and immigrant (20.3%) populations.

Laboratory diagnosis of α-thalassemia required the following studies a current hemocytometer study and the reticulocyte count (Coulter LH750 Analyzer; Beckman Coulter, Brea, CA, USA) including red cell morphology analysis. In all patients, iron deficiency was ruled out. The Hb A_2 and Hb F levels were measured by performance liquid chromatography (HPLC) (VARIANT™; Bio-Rad Laboratories, Hercules, CA, USA) and quantification of the Hb H inclusion bodies by staining with methyl violet. Hemoglobin was studied by capillary zone electrophoresis following the manufacturer's guidelines for the Sebia Capillarys Flex system using reagents provided in the Capillarys Hemoglobin (E) kit (Sebia, Norcross, GA), and HPLC analysis was performed using the manufacturer's instructions for the Bio-Rad Variant II β-thalassemia Short Program (Bio-Rad, Hercules, CA).

Following the isolation of genomic DNA with an automatic method (Biorobot® EZ1; Quiagen GmbH, Hilden, Germany), the genomic DNA was quantified by NanoDrop 1000 (Thermo Scientific, Wilmington, DE, USA).

The screening of the most frequent α globin mutation was carried out by multiplex PCR followed by reverse-hybridization with a commercial kit Alpha-Globin StripAssay (ViennaLab Diagnostic GmbH, Vienna, Austria) discarding other large deletions by Multiplex Ligation-dependent Probe Amplification (MLPA); this analysis was carried out using the MLPA Kit (SALSA MLPA KIT P140-C1 HBA; MRC Holland, Amsterdam, The Netherlands) according to the manufacturer's instructions and data from Fragment Profiler® were exported to Microsoft Excel for analysis. The molecular diagnosis was made by sequencing according to the Sanger method using in an ABI PRISM 3100 Genetic Analyzer sequencer (Applied Biosystems Foster City, Ca, USA). The α2 gene was specifically amplified with P1A (5′AGCGCCGCCCGGCCGGGCGT3′) and C3R (5′ CCATTGTTGGCACATTCCGG3′) (primers specific to the 3′UTR region of the globins α2 gene). And the α1 gene was specifically amplified with P1A (5′AGCGCCGCCCGGCC GGGCGT3′) and C2R (5′TGCGAGGAAGGCGCCATC3′) (primers specific to the 3′UTR region of the globin's α1gene). Products of the amplification were sequenced with the commercial ABI PRISMTM BigDye® Kit V1.1 Terminator Cycle Sequencing Ready Reaction Kit (PE Applied BioSystems, Foster City, CA, USA). The primers used in the sequencing were P1A (5′AGCGCCGCCCGGCC GGGCGT3′), PB (5′CCCGCCCGGACCCACA3′), and P1C (5′AGATGGCGCCTTCCTCTCAG3′).

The hemoglobinopathies described for the first time in the present work have been analyzed using the SIFT and PolyPhen-2 Software.

All our hematologic indices and clinical findings were collected with the informed, written consent of all subjects, and the study was approved by the Ethics Committee of the Hospital Clínico San Carlos, Madrid, Spain.

Results

The results of mutations responsible for α-thalassemia deletion disaggregated between heterozygous, homozygous, and double heterozygous individuals for each of the alterations are compiled in Table I. Table II collects the newly described deletions analyzed by MLPA, the probes missing, the genetic coordinates, and the hematological phenotypes. Mutations α-thalassemia non-deletion are in Table III and structural hemoglobinopathies of alpha chain globin in Table IV.

Men (48.1%) and 51.9% women were studied. The average age of the studied subjects was 30.9 years. The ethnic origin of the individuals studied revealed that the 77.5% were Caucasian, 7.4% Asian, 1% gypsies, 4.8% Maghreb, 5.3% sub-Saharan, 2.7% Latin American, 1.2% Hindus, and 0.1% Jewish from Spain.

Finally, the results in the silico analysis using the SIFT and PolyPhen-2 Software are compiled in Table V.

Discussion

This study, with 1623 individuals, is the largest one that has ever been carried out in Spain; therefore, the results obtained are entirely representative of the prevalence of alpha thalassemia deletion and non-deletion, as well as the molecular heterogeneity of the genes alpha (HBA1 and HBA2) in our country.

The Spanish geographical situation has allowed Spain not only to be a place of transit but also to suffer invasions from other western peoples such as the Phoenicians, Romans, or Arabs among others. During the 15th to the 18th centuries, due to its conquering and commercial power, Native American citizens arrived. They also received slaves from Africa and mestizos from both the American and Philippine colonies. They did not only provide us their culture and

Table I Mutations responsible of α-thalassemia deletion found in this study

Genetic diagnosis	Heterozygous cases	Homozygous cases	Double heterozygous cases	Total
$-\alpha^{3.7}/\alpha\alpha$	759	365	0	1124
--SEA/αα	68	0	0	68
--FIL/αα	19	0	0	19
$-\alpha^{4.2}/\alpha\alpha$	15	1	0	16
del of HS-40 region[a]	7	0	0	7
--THAI/αα	1	0	0	1
$--^{del\ 132\ kba}/\alpha\alpha$	*9*	*0*	*0*	*9*
$--^{del\ 28\ kba}/\alpha\alpha$	*5*	*0*	*0*	*5*
--MED/αα	4	0	0	4
--SPAN/αα	3	0	0	3
$--^{del\ 10.6\ kba}/\alpha\alpha$	*3*	*0*	*0*	*3*
$--^{del\ 33.9\ kba}/\alpha\alpha$	*3*	*0*	*0*	*3*
$--^{del\ 18\ kba}/\alpha\alpha$	*2*	*0*	*0*	*2*
--CAL/αα	2	0	0	2
$--^{del\ 37\ kba}/\alpha\alpha$	*1*	*0*	*0*	*1*
$-\alpha^{\text{del between 2.1 kb}}/\alpha\alpha$	1	0	0	1
$-\alpha^{del\ between\ 1.9\ kba}/\alpha\alpha$	*1*	*0*	*0*	*1*
$-\alpha^{6.5\ kba}/\alpha\alpha$	*1*	*0*	*0*	*1*
$-\alpha^{5.4\ kba}/\alpha\alpha$	*1*	*0*	*0*	*1*
$-\alpha^{3.7}/-\alpha^{4.2}$	0	0	10	10
$-\alpha^{3.7}/--$SEA	0	0	5	5
$-\alpha^{3.7}/--$FIL	0	0	3	3
$-\alpha^{3.7}$/del of HS-40 region[a]	0	0	3	3
$-\alpha^{3.7}/--^{del\ 132\ kba}$	*0*	*0*	*2*	*2*
$-\alpha^{3.7}/--^{del\ 10.6\ kba}$	*0*	*0*	*1*	*1*
$-\alpha^{3.7}/--^{del\ 33.9\ kba}$	*0*	*0*	*1*	*1*
$-\alpha^{3.7}/--^{del\ 8.2\ kba}$	*0*	*0*	*1*	*1*
--SEA/--FIL	0	0	1	1
Total	905	366	27	1298[b]

Italicized entries corresponds to the new mutations.

[a] Diagnosis by MLPA with SALSA P140-C1 HBA

[b] In these 1298 are included 16 patients with α-thalassemia deletion and α-thalassemia non-deletion

traditions, but they also bequeathed us their genes. Since the late twentieth century, Spain has also been a welcoming country for many immigrants, such as South Americans, Africans, and Chinese. According to a statistic set on December 31, 2014, of the Ministry of Employment and Social Security, the majority groups of foreigners residing in Spain are 2,372,490 citizens of the European Union (5.11%), 1,031,612 African (2.22%), 909,909 Latin American (1.96%), and 382,590 Asian (0.82%). In line with these data, Africans are the second primary group of foreigners living in Spain, which is consistent with the fact that both the Maghreb and sub-Saharan Africans are widely distributed in the studied population because the 10% of the people in this study came from Maghreb, sub-Saharan, although the latter does not come from endemic areas for α-thalassemia. On the other hand, Latin Americans are a minority in this study (only 2.7%) since they come from geographical areas with less prevalence of these pathologies despite being a group of foreigners very represented in Spain (≈ 1000,000 people). The majority ethnic group found in this study corresponds to the so-called Caucasians (77.5%), which are to the primary population living in Spain, followed by Asians (7.4%), came from areas endemic for α-thalassemia [5].

The average age of the subjects studied is 30.9 years; however, at stratifying the age of the subjects, it is observed that most individuals are diagnosed at an early age (25.1% of patients before 10 years of age), and this frequency is gradually falling except for a small rebound for the fourth decade of life (15.9% of cases diagnosed with ages between 31 and 40 years). This rebound coincides with the age range at which most Spanish women have their first child [6]. After giving birth to a child with microcytic anemia, the parents are advised to carry out a genetic study in search of silent carriers of alpha thalassemia.

Table II New deletions. Clinical diagnosis and phenotype-genotype relationship

Number of functional genes	Genetic diagnosis	Missing probes (from-to)	Genetic coordinates (GenBank NG_000006.1)	RBC (10^{12}/L)	Hb (g/dL)	MCV (fL)	MCH (pg)	RDW (%)	Reticulocytes (%)	Hb A_2(%)	Hb F (%)	Cases
1	$-\alpha^{3.7}/--^{\text{del 132 kb}}$	1–25	[a]	5.1	9.6	69	18.7	27.2	3.1	1.5	0.1	2
1	$-\alpha^{3.7}/--^{\text{del 33.9 kb}}$	4–22	4528–36,908	5.1	9.7	61.3	19.1	26.6	1.3	0.9	1	1
1	$-\alpha^{3.7}/--^{\text{del 10.6 kb}}$	8–26	28,168–38,786	6.5	11.3	60.7	17.4	28.1	1.5	2.2	0.7	1
1	$-\alpha^{3.7}/--^{\text{del 8.2 kb}}$	12–25	33,103–38,460	5	8.2	61.7	16.3	30.4	3.9	1.6	0.6	1
2	$\alpha\alpha/--^{\text{del 132 kb}}$	1–25	[a]	5.9	12.7	68.4	21.5	16.2	0.8	2.4	0.3	9
2	$\alpha\alpha/--^{\text{del 33.9 kb}}$	4–22	4528–36,908	5.1	11.4	71.5	22.3	16.3	0.3	2.6	0.4	3
2	$\alpha\alpha/--^{\text{del 37 kb}}$	4–25	4528–38,460	5.5	12	68.8	21.7	15.4	1	2.4	0.0	1
2	$\alpha\alpha/--^{\text{del 28 kb}}$	5–23	10,229–38,052	5.8	12.2	63.3	20.9	16.4	0.5	2.6	0.3	5
2	$\alpha\alpha/--^{\text{del 18 kb}}$	7–25	20,361–38,460	6.2	13.6	69.4	22	15.9	0.4	2.4	0.8	2
2	$\alpha\alpha/--^{\text{del 10.6 kb}}$	8–26	28,168–38,786	5.4	11.2	70.9	20.7	17.5	0.8	2.8	0.2	3
3	$-\alpha^{\text{del 1.9 kb}}/\alpha\alpha$	12–18	33,103–34,975	5.7	14.6	79.8	25.3	14.3	0.6	2.7	0.1	1
3	$-\alpha^{\text{del 6.5 kb}}/\alpha\alpha$	19–25	35,489–38,460	5.3	11.2	70.7	21.1	16.9	0.7	2.8	0.4	1
3	$-\alpha^{\text{del 5.4 kb}}/\alpha\alpha$	21–25	36,629–38,460	5.9	12.7	72.8	21.5	16.2	0.8	2.6	0.6	1
3	$-\alpha^{\text{del 2.1 kb}}/\alpha\alpha$	11–19	32,850–35,490	5.3	12.2	69.5	22.9	24.4	2.8	3	0.1	1

Diagnosis by MLPA with SALSA P140-C1 HBA and the size of the deletions corresponds to the minimum. All patients had microcytic and hypochromic anemia, to a greater or lesser degree according to the number of genes deleted. All deletions in the heterozygous state and when it only involves the loss of a gene (alpha + thalassemia) present a phenotype of thalassemia carrier and, if the deletion is extensive enough that both alpha genes have been eliminated (alpha zero thalassemia), the phenotype corresponds to a thalassemia trait. As expected, the association of these alpha zero thalassemia with an alpha + thalassemia originates Hb H disease. In all patients, iron deficiency was ruled out, the iron and ferritin levels were normal.

[a] From 92 kb upstream NG_000006.1 to position 38,460 in NG_000006.1

Table III Mutations responsible of α-thalassemia non-deletion found in this study

Genetic diagnosis	Heterozygous cases	Homozygous cases	Total	Mutation (HGVS nomenclature)
Hph	64	1	65	HBA2:c.95 + 2_ 95 + 6delTGAGG
Groene Hart	39	0	39	HBA1:c.358C > T
Plasencia	14	0	14	HBA2:c.377 T > G
Agrinio	10	2	12	HBA2:c.89 T > C
3'UTR + 778	7	0	7	HBA2:c.*46C > A
Nco	4	0	4	HBA2:c.2 T > C
T-Saudi	4	0	4	HBA2:c.*94A > G
α_1 IVS I-38 C > T	3	0	3	HBA2:c.95 + 38C > T
3'UTR + 832	3	0	3	HBA2:c.*107A > G
CD23	3	0	3	HBA2:c.70G > T
Constant Spring	3	0	3	HBA2:c.427 T > C
Tunis-Bizerte	3	0	3	HBA1:c.389 T > C
Cervantes	*3*	*0*	*3*	*HBA2:c.77G > A*
Nco A1	2	0	2	HBA1:c.2 T > C
α_2 IVS I-1 G > A	2	0	2	HBA2:c.95 + 1G > A
α_1 IVS I-1 G > A	1	0	1	HBA1:c.95 + 1G > A
PA3	1	0	1	HBA2:c.*93_*94delAA
PA2	1	0	1	HBA2:c.*92A > G
Clinic Alpha2	1	0	1	HBA2:c.184_186delAAG
Cibeles	1	0	1	HBA2:c.77G > A
α_2 IVS I-1 G > T	1	0	1	HBA2:c.95 + 1G > T
α_2 IVS II-142 G > A	1	0	1	HBA2:c.301-142G > A
Iberia	1	0	1	HBA2:c.313 T > C
α_1 IVS II-148 A > G	1	0	1	HBA1:c.301-148A > G
α_2 IVS I-78 C > G	1	0	1	HBA2:c.95 + 78C > G
α_1 IVS I-116 A > G	1	0	1	HBA1:c.96-2A > G
Marañón	*1*	*0*	*1*	*HBA2:c.94A > G*
CD19(−G)	1	0	1	HBA2:c.56delG
Gran Vía	*1*	*0*	*1*	*HBA2:c.98 T > G*
Valdecilla	*1*	*0*	*1*	*HBA2:c.3G > T*
Macarena	*1*	*0*	*1*	*HBA2:c.358C > T*
El Retiro	*1*	*0*	*1*	*HBA2:c.364_366dupGTG*
CDi ATG > GTG	1	0	1	HBA2:c.1A > G
CD22(-C)	1	0	1	HBA2:c.69delC
Adana	1	0	1	HBA2:c.179G > A
α_1 IVS I-117 G > A	1	0	1	HBA2:c.96-1G > A
Total	185	3	188[a]	

Italicized entries corresponds to the new mutations.

[a] In these 188 are included 16 patients with α-thalassemia non-deletion and α-thalassemia deletion

From the clinical point of view, only 1 severe case (hydrops fetalis), 18 cases of thalassemia intermedia owing to Hb H disease, and 1360 cases of thalassemia minor (1200 thalassemia traits and 160 thalassemia silent carriers) were recorded within the α-thalassemia clinical diagnostics. Severe cases are sporadic because prenatal diagnosis and genetic counseling in couples at risk are widespread now. On the other hand, the scarce frequency of carriers is also striking silent, but it is easy to understand that this data is strongly underestimated since these patients are characterized by an absence of clinical and lack of hematological alterations. In these cases, patients are only diagnosed because of family studies from an affected member. In this study, an understandable referral bias is observed because only the anemic, microcytic patients have been referred while microcytic patients without anemia are not.

Table IV Structural hemoglobinopathies of alpha globin chain found in this study

Genetic diagnosis	Heterozygous cases	Homozygous cases	Total	Mutation (HGVS nomenclature)
Le Lamentin	43	1	44	HBA2:c.63C > A
J-Paris	34	1	35	HBA2:c.38C > A
J-Camagüey	12	0	12	HBA2:c.424C > G
G-Philadelphia	10	0	10	HBA2:c.207C > G or 207C > A
Nunobiki	9	0	9	HBA2:c.424C > T
El Bonillo	*8*	*0*	*8*	*HBA1:c.13C > A*
J-Pontoise	6	0	6	HBA2:c.191C > A
Beziers	5	0	5	HBA1:c.300G > T
Setif	5	0	5	HBA2:c.283G > T
Burgos	*3*	*1*	*4*	*HBA1:c.193G > A*
Q-India	3	0	3	HBA1:c.193G > C
Manitoba I	3	0	3	HBA2:c.307A > C
Riccarton	3	0	3	HBA1:c.154G > A
Puerta del Sol	*3*	*0*	*3*	*HBA1:c.148A > C*
Q-Thailand	2	0	2	HBA1:c.223G > C
O-Indonesia	2	0	2	HBA1:c.349G > A
Denmark Hill	2	0	2	HBA1:c.286C > G
Watts	2	0	2	HBA2:c.226_228delGAC
Lansing	2	0	2	HBA2:c.264C > G
M-Boston	1	0	1	HBA2:c.175C > T
Titusville	1	0	1	HBA2:c.283G > A
Goya	*1*	*0*	*1*	*HBA2:c.149_172del*
Atago	1	0	1	HBA2:c.256G > T
Çapa	1	0	1	HBA1:c.284A > G
Belliard	1	0	1	HBA2:c.(171G > T or G > C)
J-Cape Town	1	0	1	HBA2:c.278G > A
Al-Ain Abu Dhabi	1	0	1	HBA2:c.56G > A
J-Medellín	1	0	1	HBA2:c.68G > A
Garden State	1	0	1	HBA2:c.248C > A
La Mancha	*1*	*0*	*1*	*HBA2:c.275 T > G*
Grady	1	0	1	HBA1:p.Thr119_Pro120insGluPheThr
Kawachi	1	0	1	HBA2:c.134C > G
Delfzicht	1	0	1	HBA1:c.30C > G
J-Broussais	1	0	1	HBA2:c.273G > T
Hasharon	1	0	1	HBA2:c.142G > C
TOTAL	173	3	176[a]	

Italicized entries corresponds to the new mutations.

[a] In these 176 are included 23 patients with simultaneous α-thalassemias and structural hemoglobinopathies

In the case of structural hemoglobinopathies, most of them are clinically silent. There were only two cases of hemoglobinopathies with low oxygen affinity, Hb Titusville and Hb Goya and one case of hemoglobin M, Hb M-Boston, which represented the only symptomatic patients. In Hb Titusville, residue 94 of α chain that plays a significant role in α1β2 interactions is affected. Individuals with this hemoglobinopathy show cyanosis [7]. Hb Goya is due to 24 bp deletion that affects eight amino acids located in helix E of the α chain of globin between which the distal His (E7) is involved. This fact relies upon the low oxygen saturation shown by the patient (87–92%) [8]. In Hb M-Boston, the distal histidine (E7) that is substituted by tyrosine is altered. This type of hemoglobin is irreversibly oxidized and, therefore, cannot provide oxygen to the tissues causing cyanosis [9, 10].

The prevalence of deletional α-thalassemia was 87.2%, an 11.7% for non-deletional α-thalassemia, and the 1.1% deletional and non-deletional α-thalassemia simultaneously.

Table V Summary of the in silico analyzes carried out by PolyPhen-2 and SIFT

Hemoglobinopathy	Polyphen-2		SIFT		Assessment
	Score	Classification	Score	Classification	
GOYA	–	–	–	–	No assessable
EL RETIRO	–	–	–	–	No assessable
BURGOS	0.016	Benign	0.46	Tolerated	Well classified
LA MANCHA	0.998	Probably harmful	0.00	It affects the function of the protein	Bad classified by both methods
PUERTA DEL SOL	0.714	Probably harmful	0.15	Tolerated	Bad classified by PolyPhen-2
EL BONILLO	0.019	Benign	0.02	It affects the function of the protein	Bad classified by SIFT
CIBELES	0.999	Probably harmful	0.00	It affects the function of the protein	Well classified
CERVANTES	0.053	Benign	0.00	It affects the function of the protein	Bad classified by PolyPhen-2
MARAÑÓN	0.975	Probably harmful	0.00	It affects the function of the protein	Well classified
VALDECILLA	0.999	Probably harmful	0.00	It affects the function of the protein	Well classified
GRAN VÍA	0.924	Probably harmful	0.00	It affects the function of the protein	Well classified
MACARENA	0.143	Benign	0.01	It affects the function of the protein	Bad classified by PolyPhen-2

The polyphen-2 score comprises values between 0 and 1, the latter value being the highest and which classifies the mutations from benign to probably harmful, respectively. In the SIFT score, the values are between 1 and 0 from least to most severe respectively (classification from tolerated to affects the function of the protein)

The prevalence of non-deletional alpha thalassemia in Spain since 2003 has gone from 7.7% to 11.7% [11] and is no longer a rare cause of thalassemia, probably due to advances and improvement of molecular biology techniques such as the MLPA, which has led into an increase in the ability to analyze large deletions.

Regarding molecular heterogeneity of the total of 1298 carriers of the deletional α-thalassemia, 905 were heterozygous carriers (−α/αα), 366 were homozygous (−α/−α), and 27 were double heterozygous. In the homozygous and heterozygous cases, the 3.7-kb deletion was the most frequent 28.1% and 58.5% of the total respectively, which agrees with the previous descriptions of other groups in Mediterranean populations such as in Italy and Greece [12]. The heterozygous cases are followed by the deletion –(SEA) and –(FIL), with 5.5% and 1.5% of the total respectively, which are widespread mutations in Southeast Asia [13] and which is our majority population group behind the Caucasians. We have identified 10 new deletions, some of them both in heterozygous and homozygous state (Table II).

A total of 188 patients with non-deletional α-thalassemia were found, of whom 185 were heterozygous, and 3 were homozygous. In this study, we have identified 36 different alterations responsible for non-deletional α-thalassemia, thus revealing the high heterogeneity in the population tested. About 75% of the α-thalassemia no deletion is due to only seven molecular alterations Hph, Hb Groene Hart, Hb Plasencia, Hb Agrinio, 3′UTR +778, Nco, and T-Saudi.

The most frequent mutation (Hph) represented 34.6% of the patients. This mutation is widely described in diverse Mediterranean populations [2, 9] such as in Lebanon (37%), Jordan (27%), or Israel (11.1%) [12]. The results highlighted the high frequency of Hb Groene Hart (20.7%) that traditionally has been associated with the North African population but was also found in the native people in this study [14]. This mutation is probably found in our community since, during the Middle Ages, the inhabitants of the Iberian Peninsula were in contact for almost 800 years with the Arabs from the Maghreb, who left offspring in the Iberian Peninsula. The Hb Agrinio accounts for 6.3% of the cases of alpha thalassemia no deletion found in our population, and the majority are in members of the gypsy community that possibly have ancestors in common with the gypsies of the east of the European continent.

Finally, it is important to note that this study has described 9 new alterations responsible for non-deletional α-thalassemia Hb Cibeles, Hb Cervantes, Hb Marañón, Hb Gran Vía, Hb Valdecilla, Hb Macarena, Hb El Retiro, 3′UTR +778, and Hb Clinic of HBA2, although each of them is in a single family [8, 15, 16].

Regarding another alteration group (structural hemoglobinopathies), a total of 176 patients were a carrier of some of these entities of α chain, and most cases were detected by chance during the quantification of glycated Hb, except for 23 that presented microcytosis. Of these 176, 173 were heterozygous, and 3 were homozygous (1 Hb Le Lamentin, 1 Hb J-Paris I, and 1 Hb Burgos) and

they did not present clinically. We have found 35 different alterations responsible for structural hemoglobinopathies of the α chain; however, the most frequent mutation (Hb Le Lamentin) represented 25% of the patients, and the second most frequent (Hb J-Paris) represented 20% of the patients. If we add the frequency of the first three variants (Hb Le Lamentin, Hb J-Paris I, and Hb J-Camagüey), they already account for half of the cases. More than 60% of the structural hemoglobinopathies are due to 5 molecular alterations Hb Le Lamentin, Hb J-Paris, Hb J-Camagüey, Hb G-Philadelphia, and Hb Nunobiki. The carriers of this Hb Nunobiki came from Seville. Tracing back to the seventeenth century, between the years 1613 and 1620, the Samurai Hasekura Rokuemon Tsunenaga led a diplomatic mission to Europe using the fluvial port of Seville. At the end of the diplomatic mission, part of the expedition stayed in Andalusia settling in Seville and are probably the ancestors of the cases currently encountered [17].

Certainly, five new alterations were described to be responsible for structural hemoglobinopathies of the α chain Hb Burgos, Hb La Mancha, Hb Goya, Hb Puerta del Sol, and Hb El Bonillo. Except Hb El Bonillo identified in 8 families only in the town of El Bonillo in Castilla La Mancha, and Hb Burgos found in three families, the rest of them were localized in only one family. [8, 16, 18].

In silico methods are used for the prediction and validation of techniques on human health. In our case, two different PolyPhen-2 and SIFT programs have been used. The in silico analysis was only carried out in those variants, not previously described, whose alteration is due to point mutations. Both programs correctly classified five hemoglobinopathies: Hb Burgos, Hb Cibeles, Hb Marañón, Hb Valdecilla, and Hb Gran Vía. Finally, with Hb La Mancha, an erroneous prediction was made by both methods. In accordance with these results, the SIFT algorithm worked better in our analyzed variants.

It is important to remember that the "in silico" studies help to assess the degree to which the new mutations are pathological or not. These tools never replace the experimental evaluation or, at least, the phenotype-genotype analysis with the available hematological data.

In this study, α-thalassemia was presented almost ten times more than structural hemoglobinopathies of the α globin chain. Presumably, this is because most structural hemoglobinopathies are clinically silent and in many cases, they have been found by chance contrasting with the severity of some forms of α-thalassemia, which is why the latter has been studied in a higher population spectrum.

On the other hand, knowing the prevalence of hemoglobin disorders is vital to ensure that the affected people receive, after diagnosis, adequate medical, psychological, and genetic counseling.

Compliance with ethical standards

The study was reviewed and approved by the Ethics Committee of the Hospital Clínico San Carlos, Madrid, Spain. All the procedures were performed in accordance with the principles of the Declaration of Helsinki.

Conflict of interest The authors declare that they have no conflicts of interest.

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