Clinical Biochemistry 63 (2019) 139–142

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Case Report

Novel nonsense mutation in the α1-globin gene [HBA1:C.49A > T] is responsible for non-deletion α-thalassemia

Paloma Ropero*, Ana Villegas, Jorge M. Nieto, Fernando A. González, Rafael Martínez

Servicio de Hematología y Hemoterapia, Hospital Clínico San Carlos, Madrid, Spain

ARTICLE INFO

Keywords:
Alpha thalassemia non deletion
Molecular diagnosis
Molecular biology

ABSTRACT

Background: In the α-thalassemia one of the less frequent mechanisms is the nonsense mutations, which generate the substitution of a triplet that encodes an amino acid for a stop codon and, therefore, protein synthesis stops prematurely. At present, 9 mutations of this type have been documented, 6 that affect the HBA2 gene and 3 that affect the HBA1 gene.

Objectives: We present a new mutation in CD16 of the HBA1 gene, where the change AAG > TAG generates a stop codon.

Methods: A 48-year-old woman from Madrid, was studied because she had maintained microcytosis without iron deficiency. Hb A2 and Hb F levels were measured by ion exchange HPLC (VARIANT II). Hemoglobin was studied by capillary zone electrophoresis and ion exchange HPLC (short program of β-thalassemia). Molecular characterization was performed by automatic sequencing of alpha globin genes.

Results: The propositus presented no abnormal hemoglobins and Hb A2 and Hb F levels were within normal limits. The molecular characterization identified the new transversion mutation HBA1: c.49 A > T, which resulted in an amino acid change of Lys > Stop at codon 16 of exon 1 in the state heterozygous [$\alpha_1$16 (A14) Lys > Stop; HBA1: c.49A > T].

Conclusion: In this new nonsense mutation, short genetic products may suffer nonsense-mediated degradation, whereas the abnormal protein will be eliminated through the proteolytic pathway mediated by ubiquitin. Regardless, the phenotype is mild. The most severe end of the clinical spectrum will probably occur when a mutation is inherited together with a mutation that results in suppression of two genes ($-/\alpha\alpha^T$ or $-\alpha/-\alpha^T$).

1. Introduction

The severity of α-thalassemia phenotypes is related to loss of synthesis of the α-globin chain. This process involves several factors, such as the number of affected genes, the degree to which the mutation decreases expression of the affected gene and the participation of the damaged gene in synthesis of the α-globin chain [1].

Most α-thalassemias are produced by genetic deletions of one or several α-genes and are known as deletion α-thalassemias. However, point mutations are also observed in critical regions of the α-genes, such as the consensus zones of the introns or the polyadenylation sequences, which can alter transcription, translation or post-translational processing of the mRNA and cause so-called non-deletion α-thalassemias [2,3].

In most cases, non-deletion α-thalassemia occurs within the hemoglobin (HB)A2 gene domain without affecting HBA1 gene expression. Thus, generally, this condition causes a greater reduction in α-globin synthesis than the reduction caused by deletion α-thalassemia [2,4].

The prevalence of non-deletion α-thalassemia is variable among populations, with a range from 30% in Israel and Jordan [5] to 20% in Iran and Lebanon [6,7] and only 3% in Brazil [8]. However, this disease presents high molecular heterogeneity. More than 100 mutations have been described that alter some processes involved in mRNA transcription through different mechanisms. Most cases are rare and privative of specific families, although several variations are more common in certain populations, such as Hph [α2 IVS-I 2-6 GAG GTG AGG > GAG G– –] in Mediterranean populations or Nco [α2 CD Initiation ATG > ACG] and T-Saudi [α2 Signal PolyA AATAAA > AATAAG] in Southeast Asia [2,9].

One of the least frequent mechanisms is nonsense mutations, which produce a substitution of a triplet coding for an amino acid with a stop codon and therefore stop protein synthesis prematurely. Currently, 9 mutations of this type are known, including 6 involving the HBA2 gene

* Corresponding author.
E-mail address: paloma.ropero@salud.madrid.org (P. Ropero).

https://doi.org/10.1016/j.clinbiochem.2018.10.015
Received 4 September 2018; Received in revised form 27 September 2018; Accepted 23 October 2018
Available online 26 October 2018

and 3 involving the HBA1 gene [9].

A new mutation in CD16 of the HBA1 gene is presented in this study, with the variation AAG > TAG producing a stop codon.

2. Materials and methods

The propositus was a 48-year-old woman from Madrid who presented maintained microcytosis without iron deficiency.

The haematological parameters were determined with an automated cell counter (Coulter LH750 Analyzer; Beckman Coulter, Brea, CA, USA). The HbA2 and HbF levels were measured by high performance liquid chromatography (HPLC) (VARIANT™; Bio-Rad Laboratories, Hercules, CA, USA). Hemoglobin was studied by capillary zone electrophoresis with the Sebia Capillarys Flex system following the manufacturer's guidelines and using reagents provided in the Capillarys Hemoglobin (E) kit (Sebia, Norcross, GA, USA). The HPLC analysis was performed with the Bio-Rad Variant II β-thalassemia Short Program following the manufacturer's instructions.

Genomic DNA was isolated using an automatic method (Biorobot EZ1; Qiagen GmbH, Hilden, Germany) and quantified with the NanoDrop 1000 (Thermo Scientific, Wilmington, DE, USA).

The most frequent α-globin mutations were discarded using multiplex PCR followed by reverse-hybridization with a commercial Alpha-Globin StripAssay kit (ViennaLab Diagnostic GmbH, Vienna, Austria), and molecular characterization was undertaken using automatic sequencing in an ABI PRISM™ 3100 Genetic Analyzer Sequencer (Applied Biosystems, Foster City, CA, USA). The α1 gene was specifically amplified with the P1A (5′-AGCGCCGCCCGGCCGGGCGT-3′) and C3R (5′-CCATTGTTGGCACATTCCGG-3′) primers; C3R is a specific primer for the 3′ untranslated region (3′UTR) of the α1-globin gene. The amplification product (947 bp) was sequenced with the commercial ABI PRISM™ BigDye® Kit V1.1 Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA). The primers used for sequencing were P1A, PB (5′-CCC GCC CGG ACC CAC A-3′), P1C (5′-AGA TGG CGC CTT CCT CTC AG-3′) and C3R.

3. Results

The haematological data are listed in Table 1. The propositus presented mild microcytosis with a MCV = 82.3 fL (85–99 fL) with maintained hypochromia MCH = 26 pg (27–34 pg) and normal reticulocytes (0.8%) (references interval 0.5–3.0). No abnormal haemoglobins were detected; the HbA2 (2.4%) and HbF (0.5%) levels were within the normal ranges (references interval HbA2 2.8–3.3 and Hb F 0.2–1.2).

Molecular characterization of the α1-globin gene by automatic sequencing identified the novel transversion mutation HBA1:c.49A > T, which resulted in an amino acid change from Lys to Stop at codon 16 of exon 1 in the heterozygous state [α16(A14)Lys > Stop; HBA1:c.49A > T]. This mutation was confirmed by sequencing of the other strand (Fig. 1),

4. Discussion

Non-deletion α-thalassemia features high molecular heterogeneity, with more than 100 different mutations described to date [9]. The present work describes a new mutation responsible for a non-deletion α-thalassemia due to substitution of A > T in CD16 of the HBA1 gene. This mutation replaces the lysine residue (AAG) at this position with a stop CD (TAG), leading to premature termination of α1-globin chain synthesis [α16(A14) Lys > Stop; HBA1: c.49A > T]. This type of mechanism is very rare among globin genes. Indeed, this mutation has not been detected in the γ-genes (A and G) and has been identified 18 times (18/913) in the β-globin gene (HBB), three times (3/126) in the δ-gene and 9 times (9/799) in the α-globin genes.

This new mutation is the fourth described in the HBA1 gene and the tenth nonsense mutation among the α-globin genes (Table 1). The first nonsense mutation in the α-globin genes was described in 1987 in a black family in the USA; no other mutation has been described since that report. These new findings are probably due to advances and refinement of molecular biology techniques, such as automation of genetic sequencing, which has improved the analysis ability.

In general, heterozygous cases of non-deletion α-thalassemia present a more marked microcytic anaemia than heterozygous cases of deletion α^+-thalassemia [10]. Additionally, within non-deletion α-thalassemia cases, patients with mutations in the HBA2 gene show a slightly more severe phenotype. This increase in severity may occur because although both HBA genes are expressed from promoter sequences with high homology and encode identical proteins, HBA2 expression is increased by 2.6-fold compared to HBA1 expression in both foetal and adult cells [11]. The most accepted hypothesis to explain this difference is polarity in the activation of the cluster by the enhancer; therefore, the closest gene is expressed at the highest level [12]. Consequently, mutations that affect the HBA2 gene would have a greater impact at the haematological level. The probability of finding a mutation in this gene would be double the probability of finding a mutation in the HBA1 gene. Indeed, any alteration in the HBA2 gene would cause the haematological parameters to be further from normality than a mutation located in the HBA1 gene, thereby facilitating detection. In fact, before this new mutation was found [$\alpha_1$16 (A14) Lys > Stop; HBA1: c.49A > T], 6 mutations in the HBA2 gene were described compared to only 3 in the HBA1 gene.

In a review of the types of nonsense mutations published to date (Table 1), we found that the differences in the haematological parameters that were more indicative of α-thalassemia (Hb, MCV and HCM) were so small that they did not justify being characterized as belonging to any individual HBA gene, even though some of the patients were African-American or Middle Eastern in descent; the haematological parameters in these ethnic groups are diminished in relation to those in Caucasians both under normal conditions and when they are carriers of α-thalassemia [13,14]. However, as expected, the smallest values corresponded to the mutations located in the HBA2 gene (Table 1). The exception was the mutation located in CD116 of the HBA2 gene identified by Liebhaber et al. in [15] when molecular biology techniques were new and specificity for the HBA1 and HBA2 genes was complicated and difficult to achieve.

Another possible explanation could be attributed to nonsense-mediated mRNA decay (NMD). NMD is a surveillance pathway that exists in all eukaryotes. Its main function is to reduce errors in gene expression by eliminating transcripts that contain premature stop codons [16]. The process of detecting aberrant transcripts occurs during mRNA translation. This model suggests that during the first round of translation, the ribosome removes exon-exon junction complexes (EJC) bound to the mRNA after splicing. NMD is activated if any of these proteins remain bound to the mRNA after this first round of translation. The most plausible theory states that the location of the last exon-junction complex relative to the termination codon usually determines whether the transcript will be subjected to NMD or not. If the termination codon is located downstream or within approximately 50 nucleotides of the final exon-junction complex, then the transcript is translated normally. However, if the termination codon is located further than approximately 50 nucleotides upstream of any exon-junction complexes, then the transcript is down regulated by NMD [17]. Based on this model, the position within the gene at which the premature stop codon (PTC) is located will be significant regardless of the location of the nonsense mutation in the HBA gene. The last exon-junction complex in the HBA gene will bind codons 99 and 100. Therefore, if a PTC is located before CD83, the aberrant mRNA will be degraded, thus avoiding expression of the truncated or erroneous α-globin chain and the possibility of producing a functionally harmful α-globin or a protein with diminished activity.

Therefore, according to this model, the closer the PTC is located to the terminal codon, the α-thalassemia phenotype is more severe.

Table 1
Summary of nonsense mutations located in alpha genes and haematological data.

Gene	Mutation	Sex/years	RBC	Hb (g/dL)	MCV (fL)	MCH (pg)	HbA2 (%)	Hb F (%)	Origin or Ethnic	Reference
HBA1	CD16	F/48	4.82	12.5	82.3	26	2.4	0.5	Spain	
HBA2	CD7	F/22	–	10.3	74.7	24.3	2.5		Iran (Middle Eastern)	[16]
	CD14	M/34	6.79	16.3	75.1	24.7	2.5	0.2	Sri-Lankan (Middle Eastern)	[19]
	CD23	F/32	4.54	12.0	84.0	26.4	2.7		Tunisia	[17]
		F/3	5.08	11.7	71.0	23.0	2.3			
	CD24	F/36[b]	5.57	10.9	65.0	19.6	2.3	0.3	Afro American (Surinam)	[18]
	CD90	F/32[a]	5.20	12.5	74.0	25.8	2.8		Middle Eastern	[19]
		M/32	5.30	13.2	74.0	26.5	2.4			
		F/26	5.30	12.6	71.6	23.7	2.6			
		F/20	5.30	12.5	71.9	23.5	2.3			
	CD116	M/54	–	14.8	84.0	27.0	2.6	0.1	Afro American	[13]
		F/43	–	11.4	79.0	24.0	2.1	0.1		
		F/37	–	12.6	80.0	25.5	2.0	0.1		
HBA1	CD14	Newborn (Cord Blood)	–	9.0	85.0	27.4			Iran (Middle Eastern)	[20]
	CD23	M/46	5.44	14.0	80.0	25.7	2.2	0.0	Italy	[21]
		M/4	4.21	10.0	72.4	23.8	2.3	0.6		
		M/1	4.39	10.3	72.0	23.5	2.2	1.8		
		F/49	5.40	14.0	78.3	26.3	2.5	0.4		
		F/22	5.18	13.0	77.3	25.1	2.7	1.5		
		M/40	6.06	15.9	80.7	26.3	3.0	0.0		
	CD99		–	17.5	77.7	25.4	2.3	0.5	Iran (Middle Eastern)	[22]
		F/29		12.4	76.2	24.5		0.4	Iran (Middle Eastern)	[16]
		M/33		14.0	71.9	23.2		0.9		
		F/25		12.0	78.1	26.2	2.5	0.0		
		F/30		13.1	76.9	25.2	3.0	0.6		
		F/29		12.7	75.5	23.9	2.0	0.3		
		M/30		15.6	77.8	25.7	2.6			
		F/28		12.7	79.2	25.6	3.2			

[a] Pregnant.
[b] $(-\alpha^{3.7}/\alpha\alpha)$.

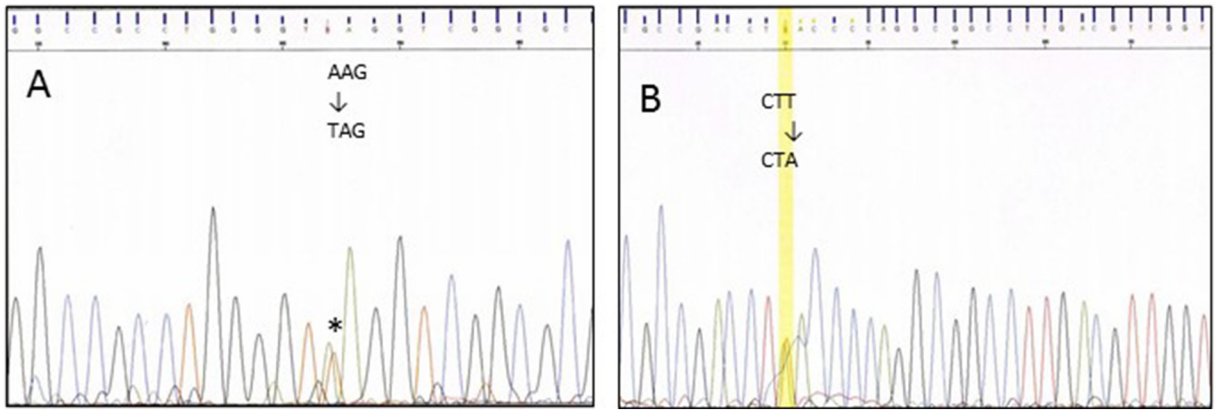

Fig. 1. Sanger sequencing analysis showing the nonsense mutation [α16(A14)Lys > Stop; HBA1:c.49A > T]. (A) Direct sequence (*A*AG > *T*AG) and (B) Reverse sequence to confirmation the change (CTT > CT*A*).

In the case of the nonsense mutation described by the present work, short genetic products may suffer nonsense-mediated degradation, whereas the abnormal protein will be eliminated through the proteolytic pathway mediated by ubiquitin. Regardless, the phenotype is mild. The most severe end of the clinical spectrum will probably occur when a mutation is inherited together with a mutation that results in suppression of two genes ($-/\alpha\alpha^{T}$ or $-\alpha/-\alpha^{T}$).

Funding information

This work has not been funded.

Compliance with ethical standards

The study was reviewed and approved by the Ethics Committee of the Hospital Clínico San Carlos, Madrid, Spain. All 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.

References

[1] A. Villegas, P. Ropero, Capítulo 10: Hemoglobinopatías y alteraciones de los eritrocitos, in: J.M. González, J.M. Medina (Eds.), Patología Molecular, Mc Graw-Hill. Interamericana, Aravaca (Madrid), 2001, pp. 171–192.
[2] C.L. Harteveld, D.R. Higgs, Alpha-thalassaemia, Orphanet J. Rare Dis. 5 (2010) 13, https://doi.org/10.1186/1750-1172-5-13.
[3] C.N. Suemasu, E.M. Kimura, D.M. Oliveira, M.A. Bezerra, A.S. Araujo, F.F. Costa, et al., Characterization of alpha thalassemic genotypes by multiplex ligation-dependent probe amplification in the Brazilian population, Braz. J. Med. Biol. Res. 44 (1) (2011) 16–22.
[4] D.R. Higgs, M.A. Vickers, A.O. Wilkie, I.M. Pretorius, A.P. Jarman, D.J. Weatherall,

A review of the molecular genetics of the human alpha-globin gene cluster, Blood 73 (5) (1989) 1081–1104.
[5] V. Oron-Karni, D. Filon, Y. Shifrin, E. Fried, G. Pogrebijsky, A. Oppenheim, D. Rund, Diversity of alpha-globin mutations and clinical presentation of alpha-thalassemia in Israel, Am. J. Hematol. 65 (3) (2000) 196–203.
[6] C.L. Harteveld, M. Yavarian, A. Zorai, E.D. Quakkelaar, P. van Delft, P.C. Giordano, Molecular spectrum of alpha-thalassemia in the Iranian population of Hormozgan: three novel point mutation defects, Am. J. Hematol. 74 (2) (2003) 99–103.
[7] C. Farra, R. Badra, F. Fares, S. Muwakkit, G. Dbaibo, I. Dabbous, et al., Alpha thalassemia allelic frequency in Lebanon, Pediatr. Blood Cancer 62 (1) (2015) 120–122.
[8] E. Borges, M.R. Wenning, E.M. Kimura, S.A. Gervásio, F.F. Costa, M.F. Sonati, High prevalence of alpha-thalassemia among individuals with microcytosis and hypochromia without anemia, Braz. J. Med. Biol. Res. 34 (6) (2001) 759–762.
[9] G.P. Patrinos, B. Giardine, C. Riemer, W. Miller, D.H.K. Chui, N.P. Anagnou, H. Wajcman, R.C. Hardison, Improvements in the HbVar database of human hemoglobin variants and thalassemia mutations for population and sequence variation studies, Nucleic Acids Res. 32 (2004) D537–D541 cited; Available from: http://globin.cse.psu.edu/hbvar/menu.html.
[10] O.M. Briceño Polacre, F.A. González Fernández, P. Ropero Gradilla, A. Ruiz, M. González, J. Briceño, M.C. Camacho, A. Villegas Martínez, No deletion alpha thalassaemia in Spain. Abnormal hematological index and molecular study, Investig. Clin. 52 (2) (2011) 111–120.
[11] Eunjung Lim, Jill Miyamura, John J. Chen, Racial/Ethnic-Specific reference intervals for common laboratory tests: a comparison among Asians, Blacks, Hispanics, and White, Hawaii J. Med. Public Health 74 (9) (2015) 302–310.
[12] E. Beutler, West Carol, Hematological differences between African-Americans and whites: the roles of iron deficiency and α-thalassemia on hemoglobin levels and mean corpuscular volume, Blood 106 (2) (2005) 740–745.
[13] S.A. Liebhaber, M.B. Coleman, J.G. 3rd Adams, F.E. Cash, M.H. Steinberg, Molecular basis for nondeletion alpha-thalassemia in American blacks. Alpha 2(116GAG > UAG), J. Clin. Invest. 80 (1) (1987) 154–159.
[14] K.E. Baker, R. Parker, Nonsense-mediated mRNA decay: Terminating erroneous gene expression, Curr. Opin. Cell Biol. 16 (3) (2004) 293–299.
[15] B.P. Lewis, R.E. Green, S.E. Brenner, Evidence for the widespread coupling of alternative splicing and nonsense-mediated mRNA decay in humans, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 189–192.
[16] N. Bayat, S. Farashi, N. Hafezi-Nejad, N. Faramarzi, M. Ashki, S. Vakili, H. Imanian, M. Khosravi, A. Azar-Keivan, H. Najmabadi, Novel mutations responsible for α-thalassemia in Iranian families, Hemoglobin 37 (2) (2013) 148–159.
[17] H. Siala, S. Fattoum, T. Messaoud, F. Ouali, N. Gerard, R. Krishnamoorthy, A novel alpha-thalassemia nonsense mutation in codon 23 of the alpha2-globin gene (GAG–> TAG) in a Tunisian family, Hemoglobin 28 (3) (2004) 249–254.
[18] P.C. Giordano, M.H. Cnossen, A.M. Joosten, C.A. Jansen, T.E. Hakvoort, M. Bakker-Verweij, S.G. Arkesteijn, P. van Delft, J.S. Waye, M.J. Bouva, C.L. Harteveld, Codon 24 (TAT > TAG) and codon 32 (ATG > AGG) (Hb Rotterdam): two novel alpha2 gene mutations associated with mild alpha-thalassemia found in the same family after newborn screening, Hemoglobin 34 (4) (2010) 354–365.
[19] A.P. Twomey, J.M. Brasch, F.R. Betheras, D.K. Bowden, A new alpha2-globin gene [codon 90 (AAG > TAG)] nonsense mutation, Hemoglobin 27 (4) (2003) 261–265.
[20] C.L. Harteveld, M. Yavarian, A. Zorai, E.D. Quakkelaar, P. van Delft, P.C. Giordano, Molecular spectrum of alpha-thalassemia in the Iranian population of Hormozgan: three novel point mutation defects, Am. J. Hematol. 74 (2) (2003) 99–103.
[21] G. Cardiero, C. Scarano, G. Musollino, F. Di Noce, R. Prezioso, S. Dembech, G. La Porta, M. Caldora, M.G. Bisconte, R. Colella Bisogno, G. Lacerra, Role of nonsense-mediated decay and nonsense-associated altered splicing in the mRNA pattern of two new α-thalassemia mutants, Int. J. Biochem. Cell Biol. 91 (Pt B) (2017) 212–222.
[22] V. Hadavi, M. Jafroodi, N. Hafezi-Nejad, S.D. Moghadam, F. Eskandari, S. Tarashohi, H. Pourfahim, C. Oberkanins, H.Y. Law, H. Najmabadi, Alpha-thalassemia mutations in Gilan Province North Iran, Hemoglobin 33 (3) (2009) 235.