

Prevalence and Genetic Profile of β -Thalassemia Associated Mutations in a Mauritanian Population

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Abstract

Objective: Although common in the Mediterranean populations, β -thalassemia are present in various other parts of the world including south Asia and Africa. This study was aimed to re-evaluate the prevalence of β thalassemia, the specific underlying β globin gene mutations and their associated haplotypes in the Mauritanian population.

Methods: β -thalassemia phenotype was investigated in 1050 unrelated Mauritians. Diagnosis was based on hematological and biochemical features. Genetic mutations were identified by direct sequencing and haplotypes analysis carried out by PCR-RFLP technique.

Results: 12 individuals had β -thalassemia phenotype giving a global prevalence of 1.14%. CD 15 G>A and CD 24 T>A represented together 50% of the detected mutations. Haplotype IX was the most prevalent in the cohort, but no mutation specific distribution was observed.

Conclusion: This study provided data on the prevalence of β -thalassemia and the underlying associated DNA changes in the Mauritanian population.

Keywords: β -Thalassemia; Mutation; Haplotype; North Africa; Mauritania

Introduction

Beta thalassemias are a group of autosomal recessive hereditary anemias characterized by the reduction or absence of synthesis in the hemoglobin beta chain. Its clinical severity is affected by genetic and environmental factors. Males and females have similar rates of disease with about 400 million reported cases, their distribution varies across the world population with specific high prevalence in peoples of Mediterranean origin, Middle East and African descent [1].

The distribution pattern of beta thalassemia in a defined group consists often of few but relatively prevalent mutations along a wider set of less represented nucleotide changes [1]. For instance, in North African populations, non-sense mutation such as codon 39 (A>T) and splice junction IVS1-1 were the most common cause of β thalassemia cases [2,3]. In sub-Saharan African populations, Substitution (A >G) at position -29 in the Tata box and IVS2-849 have been reported respectively in β^+ and β^0 U.S. black African patients [4,5].

As in sickle cell anemia, the level of anemia in beta thalassemia was also influenced by the type of the specific DNA sequence variation (haplotype) associated to the particular causative mutation [6].

In this study, we presented an updated Figure on β thalassemia prevalence in the Mauritanian population and the first data on beta-globin mutations and haplotypes among the β thalassemia disease carriers.

Materials and Methods

Prevalence of β Thalassemia

In this cross sectional study carried out from January 2015 to December 2017, hematological parameters including Hb concentration, RBC count, MCV and MCH were gathered using automated blood cell counter (Abott cell-dyn 1800, USA) from 1050 unrelated and randomly selected individuals from the population of Nouakchott, the capital city where live about 1.2 million inhabitants (27% of the total country population). Approval to this study was given by the ethics committee of the University of Sciences, technologies and Medicine, Nouakchott, Mauritania. The purpose of the study was explained to the participants and their informed consent was obtained. Parental consent obtained for the legal guardian of children below 18 years.

Subjects, aged from 2 to 72 years, were from the two main race categories of the Mauritanian population i.e. the Maures and the black African Mauritians representing respectively 80% and 20% of the global population size. The recruited patients were either voluntary blood donors at the main national center for transfusion or patients attending elective surgeries at the National Hospital the main surgical center in the country. A questioner was filled for each subject with the demographic and medical history data. Quantification of hemoglobin fractions such as HbA₂, HbF was carried out by capillary electrophoresis (Minicap Flex-Piercing, N92477, SEBIA, FRANCE).

Preliminary selection of β thalassemia subjects was based on combined MCV < 76fL MCH<24pg in subjects with microcytosis and hypochromia. Hemoglobin electrophoresis was performed only for subjects presenting normal iron status. HbF was used to identify β thalassemia patients while HbA₂ level above 3.5% indicated carrier status, asymptomatic beta thalassemia carriers were identified on the basis of a normal HbA, absence or light production of HbF (0.1-5%) level associated with HbA₂ level above 3.5%.

Thalassemia patients had 5-50% HbF and 50-95% HbF for intermediate and major thalassemia patients respectively.

Mutation Analysis

Nucleic acid variations were searched in the genomic DNA of the 12 patients identified from the random sampling and 3 already known β thalassemia patients under following. Extraction was performed from peripheral blood leucocytes by a bench top automated DNA extraction system (Arrow NA Extraction system). Preliminary screening for codon 39 and IVS1-110 mutation was carried by ARMS technique [7]. Detailed mutation search in the locus was performed by direct sequencing of PCR products carried out on a capillary 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Mutation identification was done using BioEdit database (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>).

Haplotype associated with each particular β -thal mutation was characterized on the basis of the polymorphic restriction sites cluster displayed after PCR-RFLP using specific [8].

Definition of beta thalassemia associated haplotype was based on results from polymerase chain reaction (PCR)-based restriction enzyme digestion for the beta globin gene cluster of the following polymorphic restriction sites: Hinc II 5' to ϵ , Hind III 5' to G γ , Hind III in the IVS-II 5' to A γ , Hinc II in pseudo β , Hinc II 3' to pseudo β , Ava II in β , and Hinf I 3' to β .

Haplotype not resolved by the classic nomenclature were characterized as described by Gonzalez [9]. Screening for G γ -globin promoter -158 (C>T) XmnI polymorphism was also carried out by PCR followed by XmnI restriction enzyme digestion.

Results

Out of the 1050 individuals covered by this study, twelve had hematological and biochemical parameters matching a β thalassemia phenotype giving a global prevalence of 1.14% (Table 1).

	Hb (g/dl)	VGM (fl)	HbA (%)	HbS (%)	HbF (%)	HbA ₂ (%)
SS	6-10	N	0	80-95	5-20	N
S/ β + -thalassémie	8-11	65-95	5-40	60-80	520	4-6
S/ β^0 -thalassémie	7-10	60-80	0	> 80	5-20	4-6
β thalassémie mineur	10-13	60-70	N	0	1-2	3,5-7
β thalassémie intermédiaire	7-9,5	55-70	5-45	0	10-80	3,5-7
β thalassémie majeure	< 7	50-70	0	0	90 - 95	3,5-7
PHHF	N	N	V	0	1-35	N

N: Normal; V: Variable

Table 1: Hematologic parameters and Hb composition of β -thalassemia patients and carriers

Transfusion dependent β thalassemia major with no HbA was detected in two boys six and nine years old respectively and a 50 years old woman with compound HbS/ β^0 thal who required transfusion once was also encountered. The nine other subjects, from both sexes, were all β thalassemia carriers (Table 2).

N°	Age	Gender	Hb(g/dL)	MCV (fL)	Hb A (%)	Hb F (%)	Hb S(%)	Hb A2(%)	Phenotype
Patients									
1	6	M	6,6	59,2	0	97,9	0	2,1	β major
2	9	M	5	60,9	0	98	0	2	β major
3	50	F	8	75	19,9	5,1	70,2	4,8	HbS/ β^+ thal
Carriers									
4	20	F	11,3	68,1	95,7	0	0	4,3	β minor
5	13	M	12,7	73,7	95,9	0	0	4,1	β minor
6	15	M	8,6	58,1	95,4	0	0	4,6	Bminor
7	14	F	12,8	77,3	95	0,2	0	4,8	Bminor
8	14	M	13.1	67	95,1	0,4	0	4,5	Bminor
9	30	F	12	79	95,6	0	0	4,4	Bminor
10	21	F	10,1	58,6	92,9	2,1	0	5	Bminor
11	35	M	12,7	76	92,2	2,9	0	4,9	Bminor
12	12	M	12,8	69,3	95,8	0	0	4,2	Bminor

Table 2: Hematologic parameters and Hb composition of β -thalassemia patients and carriers

Looking at the causative mutation, we found no codon 39 or IVS-110 among these patients. The two boys, one black African and one Maure, were both homozygote for the causative mutation, e.i. CD 61 A>T and IVS1-2 (T >G) respectively. CD 24 T>A mutation was detected in the black woman with compound HbS/ β^+ thal.

In the carriers group, Substitution CD 15 G>A, found in four individuals in heterozygous status, was the most encountered β thalassemia nucleotide change followed by CD 24 T>A present, also in trait thalassemia, in two subjects. IVS1-2 (T >G), -29 A>G and -30 T>A were identified only once each (Table 3).

N°	Ethny	Mutation	Alleles	Haplotype	XmnI
1	Black	CD 61 A>T	2	B	-
2	Maure	IVS1-2 (T >G)	2	IX	+
3	Black	CD 24 T>A	1	IX	+
4	Maure	CD 15 G>A	1	III	+
5	Maure	CD 15 G>A	1	III	+
6	Maure	CD 15 G>A	1	B	-
7	Maure	CD 15 G>A	1	ND	-
8	Black	CD 24 T>A	1	V	-
9	Black	CD 24 T>A	1	IX	+
10	Maure	IVS1-2 (T >G)	1	IX	+
11	Black	-29 A>G	1	IX	+
12	Maure	-30 T>A	1	III	+

Table 3: Haplotypes distribution in β -thalassemia carriers

Mutations ethnic distribution (Table 2) showed that CD 15 G>A, IVS1-2 (T >G), -30 T>A were found only in the Maures group while CD 24 T>A, CD 61 A>T and -29 A>G were found in the black Africans.

The polymorphic restriction sites cluster analysis showed the predominance of two known haplotypes in the cohort. Five individuals displayed haplotype IX (-+----) while haplotype III (-+----) was observed in three subjects (Table 2). No ethnic association was observed. For instance, haplotype IX, the predominant cluster, was identified both in the Black and Maures categories respectively presented with CD 24 T>A and IVS1-2 (T >G). Three individuals showed non classic haplotype. Two of them were determined as haplotype B (----+--).

When we used the American classification.

-158 T>C XmnI site was positive in 8 out of the 12 β thal mutation carriers. This polymorphism was detected both in patients with homozygous or heterozygous beta thalassemia. No mutation specific distribution of this site was observed (Table 2).

Discussion

We have shown a prevalence of 1.14% for all forms of β -thalassemia in the cohort screened. Deyde *et al.* have presented an overall frequency of 2.57% in a Mauritanian cohort of 750 individuals [10], in his study, mutation search was carried out only in patients

presenting microcytic, hypochromic anemia with normal iron status and increased Hb A2 level, as iron deficiency could lower A2 level we may, consequently, have missed β thal carriers, with apparent normal or decreased A2, as they have iron deficiency. These individuals were indeed not screened for mutation analysis. As a result the global prevalence of beta thal carriers found here may have indeed slightly been underestimated here. The study of Deyde *et al.* did not mention the iron status of the screened subjects. However, both percentages are close to the range of 1.5-3% reported in the populations of Morocco, Algeria and Tunisia [3,11,12].

The mutation spectrum we observed revealed two β chain preventing formation allele variants i.e. IVS1-2 (T >G) splice junction in the white Maure group and the non-sense codon CD 61 A>T in the black Africans. Both point mutations caused transfusion-dependent β thalassemia major (β^0). Their occurrence here was in accordance with the reported specific distribution respectively in the North Africans and the blacks [4]. A similar pattern of allelic heterogeneity was observed in β thalassemia minor cases where CD 15 G>A was found only in the Maures group while CD 24 T>A and -29 A>G were present only in the black Africans.

These results concord with previous studies [13,14] showing that the prevalence and ethnic distribution of different biomarkers reflected the heterogeneity of the Mauritanian population made of two main racial groups. The Moors sharing the same Arab-Berber origin with the North African populations and the Mauritanian black Africans group composed of three ethnies (Pulhar, Soninké and Wolofs), all from the same sub-Saharan black African descent.

γ^G -158 (C>T) polymorphism causes a hereditary continuous production of τ chain and as a result the persistence level of HbF (HPFH) in adult life. As it does with sickle cell anemia, the most dominant hemoglobin disorder in African populations, it seems also to influence the β thalassemia phenotype [15]. In this study, Xmn I polymorphism was detected in two thirds of the patients both in β^0/β^0 and heterozygous groups. Although we did not assess here the presence of this polymorphism in correlation with HbF expression, others studies have shown that HbF levels were significantly higher in individuals with XmnI than in controls without [16,17]. Besides, Mean red cell size (MCV) and mean hemoglobin content per red cell (MCH) were positively correlated with HbF level suggesting that increased HbF level improved the erythropoietic environment significantly in β -thalassemia.

One limitation of this study was the number of beta thalassemia patients used in this study for the mutation search. Indeed, as indicated from the low prevalence, we could not find a number of patients allowing a statistically significant analysis of the data presented here.

Conclusion

The results presented here on β thalassemia carrier prevalence, the genetic mutations and haplotypes associated, provides the first global clinical and genetic description of hemoglobin disorder in the Mauritanian population.

Conflict of Interest

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

References

1. Global Burden of Disease Study 2013. Collaborators (2015) Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 386: 743-800.
2. Hardison RC, Chui DH, Riemer C, Giardine B, Lehvälaiho H, et al. (2001) Databases of human hemoglobin variants and other resources at the globin gene server. *Hemoglobin* 25: 183-93.
3. Jouini L, Sahli CA, Laaouini N, Ouali F, Youssef IB, et al. (2013) Association between clinical expression and molecular heterogeneity in β -thalassemia Tunisian patients. *MolBiol Rep* 40: 6205-12.
4. Agouti I, Badens C, Abouyoub A, Levy N, Bennani M (2008) Molecular basis of beta-thalassemia in Morocco: possible origins of the molecular heterogeneity. *Genet Test* 12: 563-8.
5. Thein SL (2013) The molecular basis of β -thalassemia. *Cold Spring Harb Perspect Med* 3: a011700.
6. Antonarakis SE, Irkin SH, Cheng TC, Scott AF, Sexton JP, et al. (1984) beta-Thalassemia in American Blacks: novel mutations in the "TATA" box and an acceptor splice site. *Proc Natl Acad Sci USA* 81: 1154-8.
7. Wajcman H, Riou J (2009) Globin chain analysis: an important tool in phenotype study of hemoglobin disorders. *Clin Biochem* 42: 1802-6.
8. Old J (2010) Henderson S. Molecular diagnostics for haemoglobinopathies. *Expert Opin Med Diagn* 4: 225-40.
9. Antonarakis SE, Kazazian HH Jr, Orkin SH (1985) DNA polymorphism and molecular pathology of the human globin gene clusters. *Hum Genet* 69: 1-14.
10. Gonzalez-Redondo JM, Stoming TA, Lanclous KD, Gu YC, Kutlar A, et al. (1988) Clinical and genetic heterogeneity in black patients with homozygous beta-thalassemia from the southeastern United States. *Blood* 72: 1007-14.
11. Deyde VM, Lo BB, Khalifa IO, Ly B, Ball A (2002) Epidemiological profile of hemoglobinopathies in the Mauritanian population. *Ann Hematol* 81: 320-1.
12. Labie D, Bennani C, Beldjord C (1990) Beta-thalassemia in Algeria. *Ann N Y Acad Sci* 612:43-54.
13. Fattoum S (2006) Hemoglobinopathies in Tunisia. An updated review of the epidemiologic and molecular data. *Tunis Med* 84: 687-96.
14. Veten FM, Abdelhamid IO, Meiloud GM, Ghaber SM, Salem ML, et al. (2012) Hb S [β 6(A3)Glu→Val, GAG>GTG] and β -globin gene cluster haplotype distribution in Mauritania. *Hemoglobin* 36: 311-5.
15. Hamed CT, Bollahi MA, Abdelhamid I, Mahmoud MAM, Ba B, et al. (2012) and ethnic distribution of ABO and Rh(D) blood groups in Mauritania: results of first nationwide study. *Int J Immunogenet* 39: 151-4.

16. Agouti I, Badens C, Abouyoub A, Khattab M, Sayah F, et al. (2007) Genotypic correlation between six common β thalassemia mutations and the Xmn I polymorphism in the Moroccan population. Hemoglobin 31: 141-9.
17. Chinelato IS, Carrocini GCS, Bonini-Domingos CR (2011) XmnI polymorphism frequency in heterozygote beta thalassemia subjects and its relation to Fetal hemoglobin levels Rev Bras Hematol Hemoter 33: 483-91.

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