The predominance of codon 39 (c>t) mutation of *HBB* gene in a portion of the Algerian population (Northeast Algeria)

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Abstract

This study was planned to determine the frequency of β-thalassemia mutations in Batna region (Northeast Algeria). Nineteen blood samples of clinically thalassemic children patients were collected from Department of Pediatrics, University Hospital of Batna. We carried out the molecular genetics of beta globin gene by the method of minisequencing using Snapshot™ kit (Applied Biosystems) in search of the four most common *HBB* genetic variants including three β-thalassemia mutations: codon 39(C>T) (*HBB*: c.118C>T), IVSI-110(G>A) (*HBB*: c.93-21G>A), and IVSI-1-2(T>G) (*HBB*: c.92+2T>G), as well as the hemoglobin S variant (*HBB*: c.20A>T). We used direct DNA sequencing to detect the rare mutations of beta-globin gene. We have revealed the presence of four different *β*-globin gene mutations responsible for *β*-thalassemia in Batna region. According to our results, the nonsense mutation at codon 39 (C>T) is the most frequent mutation type in our province, the same as other geographical regions of Algeria. It is followed by codon 54(-T), detected in a second Algerian family (the proband was homozygote), and the first association of Hb Knossos: codon 27 (G>T) allele with codon 39 (C>T) in the Algerian population. Here we reportws also the association of codon 39(C>T) with IVS-I-110 (G>A). Our preliminary results show the predominance of codon 39 (c>t) mutation of *HBB* gene in Batna region.

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Introduction

β-thalassemia is a recessive monogenic disorder encountered worldwide with a higher prevalence among Mediterranean, Middle Eastern and Indian populations.[1] The disease is due to mutation in β- globin locus. Where about 300 alleles have been reported.[2] Numerous disorders of the β-globin chain of hemoglobin lead to different disease phenotypes.[3,4] Of these, β-thalassemia is a subset of the β-hemoglobinopathies characterized by a hereditary anemia with a wide phenotypic spectrum that can have significant morbidity and mortality.[4,5] The β-thalassemia exhibit a range of severities, each corresponding to an absence or reduction of β-globin protein synthesis. These β-thalassemia phenotypes are related to the myriad mutations that affect the β-globin gene (*HBB*) on chromosome 11p15.5, and different populations have their own mutation spectrum.[6] In Algeria, the frequency of β-thalassemia gene is 3%;[7] these diseases are a real public health problem often compounded by rate inbreeding of the population (30-32%).[8] Previous investigations have disclosed a high molecular heterogeneity of β-thalassemia.[9,10] This study aims to describe the mutation spectrum from a sample of β-thalassemia patients and from β-thalassemia carriers. In addition the present study was performed to assess the usefulness of the mini-sequencing technique as an alternative strategy for genetic diagnosis of *HBB* gene disorders as a screening technique for the detection of unknown β-globin gene mutations in samples of known β-thalassemia patients.

Materials and Methods

This study was realized in β-thalassemia homozygous children and from β-thalassemia carriers (age brackets 4 to 16 year old of male and female sex). These subjects are from the region of Batna, cared in the pediatric ward of the University Hospital Batna. Venous blood samples of 2.5 mL volume were drawn from the study subjects and were collected in EDTA anticoagulant containers. Blood withdrawals were performed a few minutes before

the regular blood transfusion for patients homozygous whereas heterozygous children the sampling is performed during family investigations.

DNA extraction

The molecular analysis of the *HBB* gene was carried out after taking informed written consent from all the parents of the minors. Genomic DNA was extracted from peripheral blood leukocytes using the FlexiGene-DNA Kit (Cat # 51206; Qiagen Inc., Valencia, CA, USA) and stored at 4°C.

Minisequencing reaction of *HBB* gene

The minisequencing assay was developed for the detection of the four most common *HBB* genetic variants including three β-thalassemia mutations: codon 39(C>T) (*HBB*: c.118C>T), IVSI-110(G>A) (*HBB*: c.93-21G>A) and IVSI-1-2(T>G) (*HBB*: c.92+2T>G), as well as the hemoglobin S variant (*HBB*: c.20A>T). To detect these four mutations, an allele specific PCR was performed, followed by highly multiplexed minisequencing reaction. The specific primer sequences of the *HBB* gene and PCR conditions are available upon request. Polymerase chain reaction products were purified using QIAquick PCR Purification by Kit (Qiagen Inc.). Purified fragments were used as template in a primer extension reaction containing the mutation-specific primer cocktail (Table 1).

For the extension reaction, we used the SNaPshot Multiplex Kit (Applied Biosystems, Foster City, CA, USA), following manufacturer's instructions. After extension, the samples were treated with shrimp alkaline phosphatase according to the manufacturer protocol.

Multiplex minisequencing products were resolved by automated capillary electrophoresis ABI PRISM 310 Genetic Analyzer (Applied Biosystems). Briefly, 12 mL of HiDi™ formamide and 0.5 mL size GeneScan 120 LIZ-calibrator (Applied Biosystems) were added to 1 mL of multiplex minisequencing product. The mixture was denatured at 95°C for 3 min. next transferred to ice for 2 min. and loaded on an ABI PRISM® 310 Genetic Analyzer capillary.

Direct DNA sequencing

If the minisequencing technique presents a snapshot normal, we use the direct DNA sequencing. The *β-globin* gene was amplified using couples of primers: *HBB* F: 5'-CTG ACA CAA CTG TGT TCA CT-3'and *HBB* R: 5'- TTC ACC TTA GGG TTG CCC -3'(exon 1). The fragment size of primers product was 355 bp.

The β-thalassemia mutation was identified by automated sequence analysis performed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems) using the fluorescent dideoxy-termination method (Big Dye-Terminator Cycle Sequencing Kit; Applied Biosystems).

Results

Here we apply the minisequencing technique as an alternative strategy for genetic diagnosis of *HBB* gene disorders in children with β-thalassemia, provided by the pediatrics department, Hospital Batna (Northeast of Algeria). We chose this method as it allows quick search of the 4 most common and frequent mutations of the *HBB* gene. The GeneScan electropherograms of our subject's samples after multiplex minisequencing primers are shown in Figures 1 to 5 and the results of direct DNA sequencing are shown in Figures 6 and 7.

Table 2 shows the results of molecular diagnosis for the 38 chromosomes from 9 individuals with beta-thalassemia trait and 10 children with beta-thalassemia major analyzed with 4 different beta-thalassemia mutations. This study confirms the observations that the frequency of several mutations varies from one ethnic group to another. The four different β-thalassemia mutations have been identified in this study, were codon 39 (C>T) the most frequent β-thalassemia mutations. In addition, two genetic variants without disease association, the polymorphisms codon 39 (C>T) and IVS-I-110 (G>A), a first association of Hb Knossos: *HBB*: c.82 G>T with *HBB*: c.118 C>T mutation causes thalassemia homozygous in the Algerian population, was found in one subject.

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Table 1. Primers for multiplex minisequencing analysis.

Investigated mutations*	Minisequencing primers (sequences in 5' 3' direction)
HbS (*HBB*: c.20A>T)	T(45) ATG GTG CAC CTG ACT CCT G
IVS-I-2 (T G) (*HBB*: c.92+2T>G)	T(55) GTG AGG CCC TGG GCA GG
IVS-I-110 (G A) (*HBB*: c.93-21G>A)	T(65) ACT GAC TCT CTC TGC CTA TT
Codon 39 (C T) (*HBB*: c.118C>T)	T(75) GTG GTC TAC CCT TGG ACC

*The variants are described using Human Genome Variation Society nomenclature.

Table 2. Spectrum of beta-thalassemia mutations in northeast Algeria (Batna).

HBB mutation name or variant	Phenotype	Localization at *HBB*	Genomic variation (HGVS)	No. of alleles	%
Codon 39 (C>T)	β0	Exon 2	c.118C>T	32	84.21
IVS-I-110 (G>A)	β+	Intron 1	c.93–21G>A	3	7.89
Codon 27 (G>T) Hb Knossos	β+	Exon 1	c.82G>T	1	2.63
Codon 54(-T)	β0	Exon 1	c.165delT		5.26

Codon 54(-T) mutation was found in one subject who was homozygous for this molecular lesion.

Discussion

This is the first study investigating the molecular level basic of β-thalassemia in the region of Batna (East of Algeria). We conducted the identification and characterization of the molecular basis of β-thalassemia among children born in BATNA region. This type of disease is very rare in this region compared to the size of its population.

The β-thalassemia in Algeria with its frequency and severity presents a public health problem, mainly in transfusion support.[11] Accordingly,[12] the prevalence of β-thalassemia allele in North Africa, would increase from west to east Mediterranean countries (Morocco 0.94%, 1.4% of Algeria, Tunisia 3% and Egypt 4.5%).[13,14]

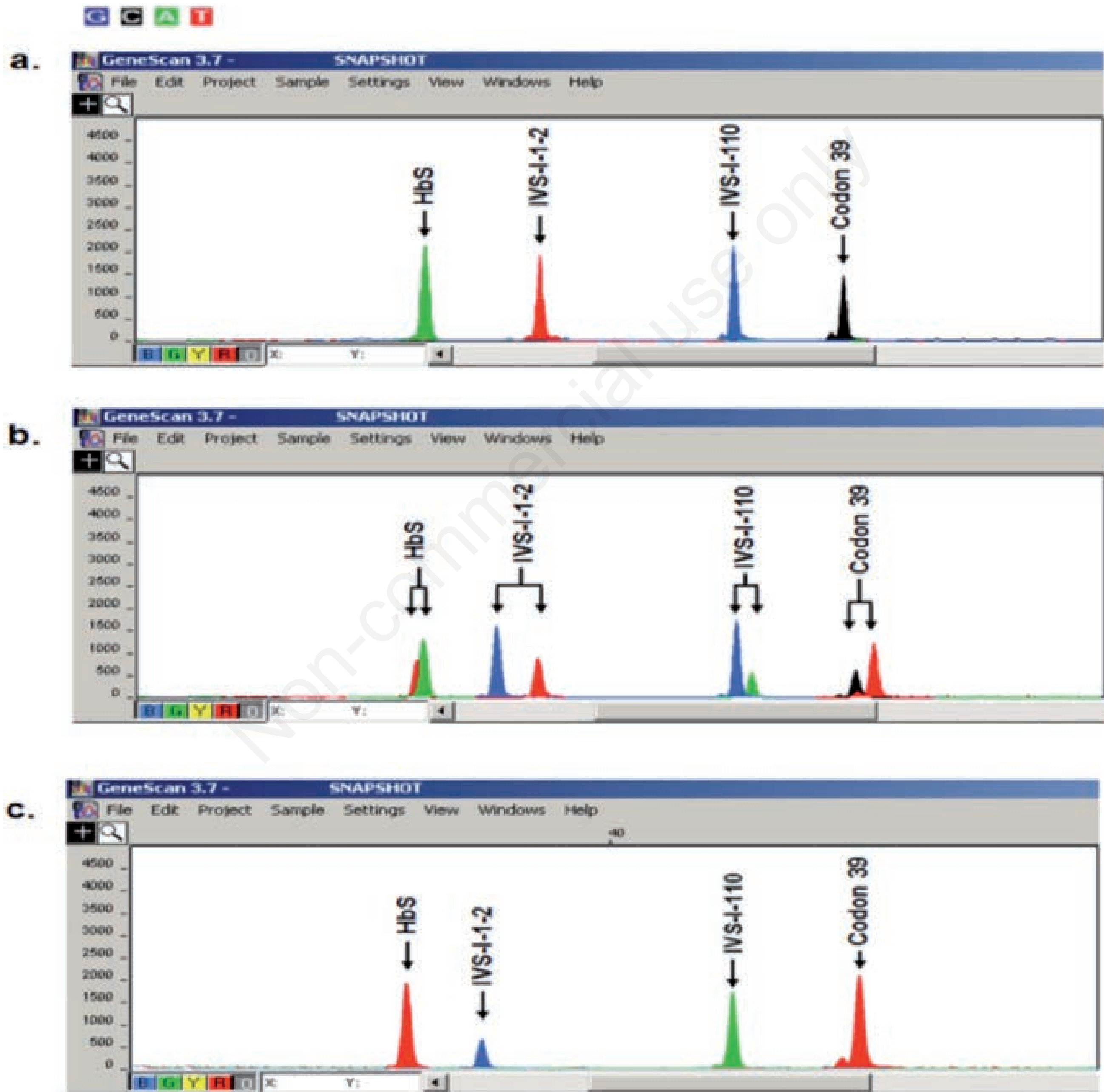

Figure 1. GeneScan analysis of the multiplex minisequencing reaction. Electropheregrams: a) shows the analysis results of a *HBB* wild-type sample; b) illustrates the pattern of peaks for all mutant positions in the heterozygous state; c) illustrates the pattern of peaks for all mutant positions in the homozygous state. The red color peaks represented the normal alleles and the green color peaks represented the mutant alleles.

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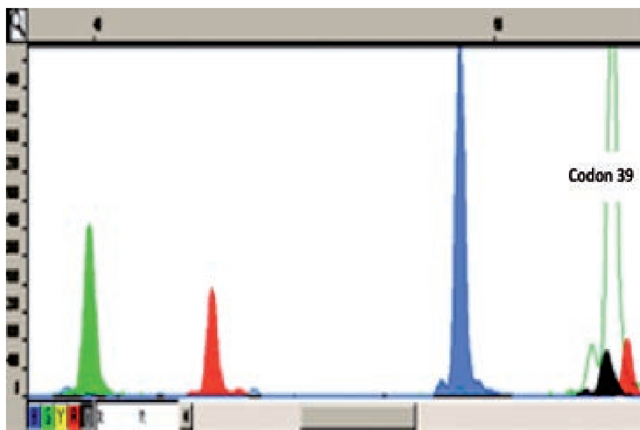

Figure 2. Electropherogram showing a peak for codon 39(C>T) mutation in the heterozygous state.

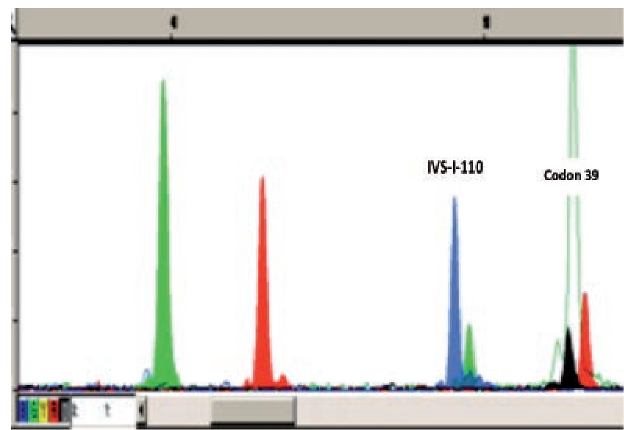

Figure 4. Electropherogram showing a peak for codon 39(C>T) mutation and IVS-I-110 (G>A) in double heterozigosity state.

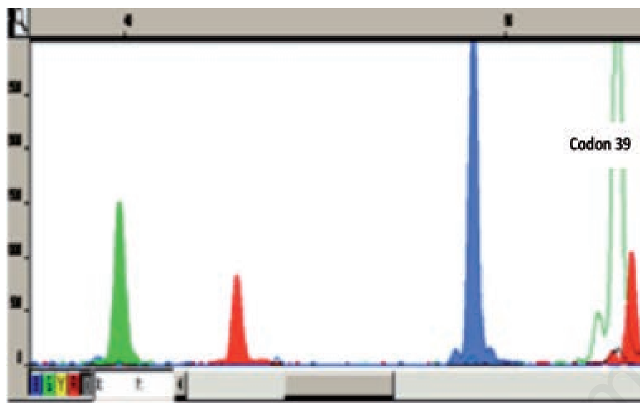

Figure 3. Electropherogram showing a peak for codon39(C>T) mutation in the homozygous state.

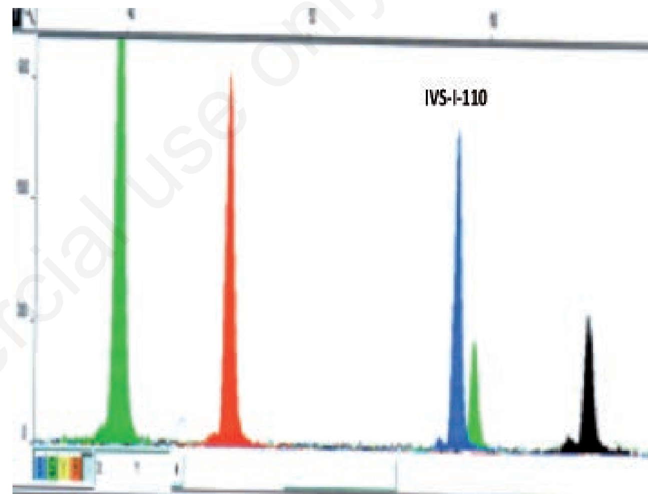

Figure 5. Electropherogram showing a peak for IVS-I-110 (G>A) in the heterozygous state.

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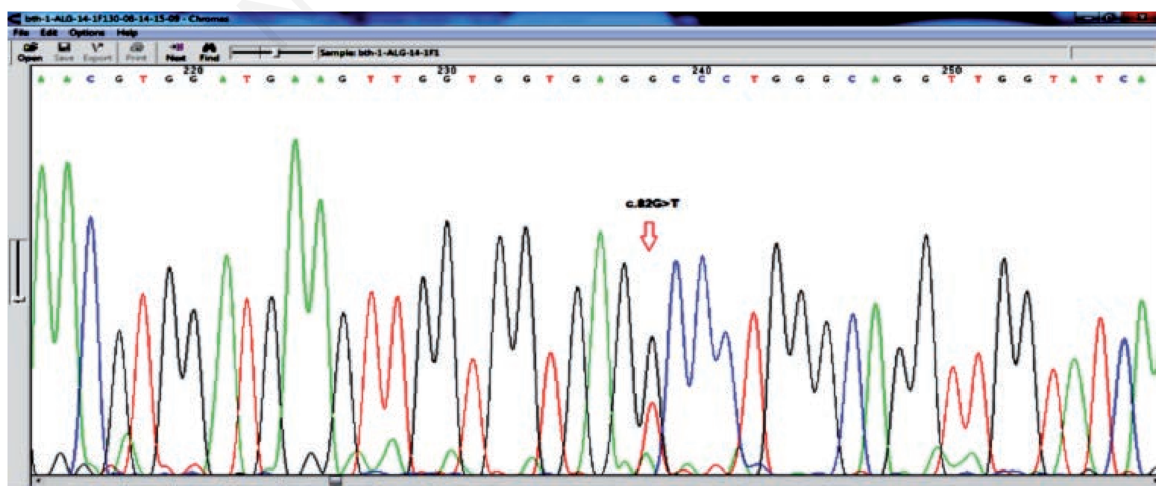

Figure 6. Part of electropherogram obtained by sequencing the genomic DNA from the beta-thalassemic patient showing the presence of codon 27 (G>T) mutation (Hb Knossos).

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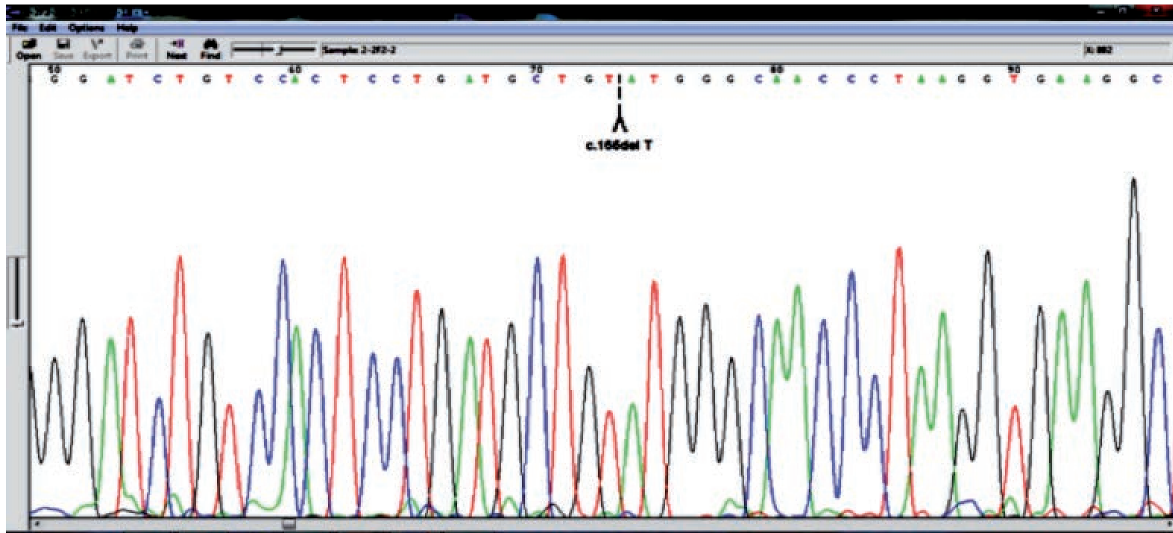

Figure 7. Part of electropherogram obtained by sequencing the genomic DNA from the homozygous beta-thalassemia patient showing the presence of c.165delT mutation.

A great heterogeneity of the molecular defects at the origin of the β-thalassemia is detected in Algeria and the number of mutations of β-thalassemia is of 25 molecular lesions.[11] On the other hand, the Algerian population is characterized by four dominant mutations, which represent over 80% of β-thalassemia alleles. These are the mutation nonsense codon 39 C→T; IVS-I-110 substitution G→A; framshift the codon 6 (-A) and mutation IVS-I-1 G→A (16).

The nonsense mutation codon39(C>T) is widespread in Algeria with a frequency 25.94%,[11] 27.6%[15] and is more common in the west and decreases in center to be predominant in the East.[16]

IVS-I-110 although this mutation was discovered in 1981[17,18] and is caused by the replacement of a guanine by adenine in the consensus sequence, located in the first intron of the β-globin gene.[19] The IVS-I-110 mutation found in Turkey,[20] represents 40% beta thalassemia alleles in our study, it is predominant in central Algeria and located in a low frequency in the west (16%).[15] This contribution is consistent with the extent of the Ottoman Empire between the 16th and 19th century. In Tunisia, it is (21%)[21] and Egypt (26%).[22] It is rare in Morocco (3.2%).[23] For Codon 54(-T): c.165delT; this is the second Algerian family which carries this mutation. The deletion of T from codon 54 result in framshift with a nonsense codon at codon 60 (TGA) and premature termination of translation,[24] this mutation is detected for the second time in the Algerian population. In this study, we detect a rare hemoglobin variant caused by a mutation in *β-globin* gene, HBB: c.82G>T: Codon 27 GCC>TCC (Ala-Ser), Hb Knossos, which produces the classical phenotype of intermedia β-thalassemia in association with *HBB*: c.118C>T) mutation causes β-thalassemia homozygous in an Algerian children patient with transfusion depended β-thalassemia.[25,26] Hb Knossos (fl 27 (B9) Ala-Ser) in the heterozygous state has been recently recognized as the underlying abnormality in atypical microcytosis[25,27] First identified in a family from Crete, Hb Knossos was discovered soon afterwards in two families from northeast Algeria and in a family from the French West Indies.[28,29]

Conclusions

In this study, we used the minisequencing assay as a rapid screening procedure to identify four most common *HBB* genetic variants including three beta-thalassemia mutations and direct DNA sequencing to detect the rare mutations of beta-globin gene. Our data show the predominance of codon 39 (c>t) mutation of *HBB* gene in Batna region, despite the heterogeneity of the beta-thalassemia mutations in Algerian population. Four different β-thalassemia mutations have been identified in the Batna population. Codon 39 (C>T) is the most frequent mutation type in our province, followed by codon 54(-T), and the first association of Hb Knossos: codon 27 (G>T) with codon 39 (C>T) in Algerian population. Here we reported also the association of codon 39 (C>T) with IVS-I-110 (G>A). Although the molecular defects at the origin of this pathology are of extreme diversity, each population is characterized by a group of 4 to 5 mutations that is specific to it.

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