# A comprehensive review of the prevalence of beta globin gene variations and the co-inheritance of related gene variants in Saudi Arabians with beta-thalassemia

*Mousa A. Alaithan,* BSc, *Sayed AbdulAzeez,* MSc, PhD, *J. Francis Borgio,* MSc, PhD.


## ABSTRACT

البيتا ثلاسيميا هو اختلال جيني يحدث بسبب وجود طفرات في الجين المكون للبيتا هيموجلبين (HBB). تعتبر السعودية واحدة من أكثر الدول في انتشار المصابين بهذا المرض وخصوصاً في المنطقة الشرقية. هذه الدراسة شُكِلت في محاولة لتجميع كل الطفرات المسجلة وجعلها أساس نواة أولية لدراسات أكثر تفصيلية في تسمية وإحصاء انتشار الطفرات الجينية المسببة لمرض البيتا ثلاسيميا على المستوى الوطني. تستحوذ الطفرتان IVSI–5 (G>C) و Cd 39 (C>T) النسبة الأعلى في الانتشار بين جينات المصابين بهذا المرض من بين 42 طفرة وراثية مُكتشفة لمصابين سعوديين بمرض البيتا ثلاسيميا. لوحظ بشكل شائع عند المصابين السعوديين انتشار طفرات لجينات مختلفة موروثة بمصاحبة طفرات جين للبيتا هيموجلبين HBB كـ ATRX، AHSP، HBA12، HBA2، HBA1، وKLF1. يهدف هذا النوع من الدراسات إلى إعداد دراسات على المستوى الوطني في الطبيعة الجزيئية لمرض البيتا ثلاسيميا و يوصي بتوحيد جهود مراكز البحث الجينية الطبية المنتشرة في مناطق مختلفة داخل المملكة لتشكيل شبكة من قاعدة بيانات موحدة تحوي سجلات ونتائج فحوص ماقبل الزواج للمساهمة في تطوير استراتيجية أكثر فاعلية في تقليل نسبة المرض المرتفعة داخل السعودية والبحث في أفضل الخطط العلاجية له.

Beta-thalassemia is a genetic disorder that is caused by variations in the beta-hemoglobin (*HBB*) gene. Saudi Arabia is among the countries most affected by beta-thalassemia, and this is particularly problematic in the Eastern regions. This review article is an attempt to compile all the reported mutations to facilitate further national-level studies to prepare a Saudi repository of *HBB* gene variations. In Saudi Arabians, IVSI-5 (G>C) and Cd 39 (C>T) are the most prevalent *HBB* gene variations out of 42 variations. The coinheritance of *HBB* gene variations with *ATRX*, *HBA1*, *HBA2*, *HBA12*, *AHSP*, and *KLF1* gene variations were observed to be common in the Saudi population. National surveys on the molecular nature of hemoglobinopathies should be set up through collaborations between research centers from various regions to create a well-documented molecular data bank. This data bank can be used to develop a premarital screening program and lead to the best treatment and prevention strategies for beta-thalassemia.


*Saudi Med J 2018; Vol. 39 (4): 329-335*
*doi: 10.15537/smj.2018.4.21360*

*From the Department of Genetic Research, Institute for Research and Medical Consultations, Imam Abdulrahman Bin Faisal University, Dammam, Kingdom of Saudi Arabia.*

*Address correspondence and reprint request to: Dr. J. Francis Borgio Department of Genetic Research, Institute for Research and Medical Consultation, Imam Abdulrahman Bin Faisal University, Dammam, Kingdom of Saudi Arabia. E-mail: fbalexander@iau.edu.sa/borgiomicro@gmail.com*
*ORCID ID: orcid.org/0000-0001-7199-1540*

Hemoglobinopathies are disorders that affect the hemoglobin molecule, which carries oxygen from the lungs to the rest of the body. Hemoglobin is a protein that consists of 2 α-like type and 2 β-like type chains. A variation on the globin genes can lead to an error in the production of the coded chains, which cause hemoglobinopathies, such as thalassemia and sickle cell diseases. Beta-thalassemia is a prominent recessive genetic disorder that is usually caused by single-point variations (formerly called mutations) or deletions that are inherited from parents who are carriers or affected by the disease variants. Sequence variations that completely prevent the synthesis of the β-globin chain of the hemoglobin molecule are termed $\beta^0$ variants; however, variations that reduce protein expression or change the molecular nature of the protein chains are called $\beta^+$ variants.[1-3] The main objective of this review is to compile all previously reported β-hemoglobin gene

(*HBB*) variants from the Saudi population to create a national *HBB* variation reference source for future research and clinical applications.

Saudi Arabia is among the Arab countries most affected by β-thalassemia. As a result, the Ministry of Health initiated a national program that provides premarital screening and genetic counseling to control the high prevalence of hemoglobinopathies by spreading awareness among nationals.[4-6] The main reason for the high prevalence is the high percentage of consanguineous marriages among Saudis.[7] The Ministry of Health requires that Saudis take a test if they want to get married. This screening test involves taking blood samples in ethylenediaminetetraacetic acid (EDTA) vacutainers from Saudis in designated centers around the country. The samples are then sent to one of the 125 specialized laboratories where hemoglobin electrophoresis and other standard blood testing is carried out.[6-8] Those who have β-thalassemia or are carriers of the disease have a hemoglobin $A_2$ ($HbA_2$) level above 3.5% and a low mean corpuscular volume (MCV) with no appearance of iron-deficiency anemia.[9,10] However, the use of the $HbA_2$ level as a diagnostic marker for the identification of carriers or the β-thalassemia trait is debatable.[11] Therefore, a precise country-wide data bank based on national-level research is needed to develop error-free molecular diagnostic methodologies.

***Prevalence of β-globin mutations.*** Studies on the molecular basis of thalassemia have been performed in a very limited number of Saudi Arabians. Except for the national study that was conducted in the 1980s and 1990s by El-Hazmi et al[4,12,13] there has been no research on the molecular basis of β-thalassemia or on mutations that cause the disease in any region other than the Eastern Province and Makkah. In the last 20 years, only 6 studies were conducted on the molecular basis of β-thalassemia to determine the prevalence of β-globin gene mutations, whereas a few other studies reported only one or 2 *HBB* variations. Out of these 6 prevalence studies, 4 were conducted in the Eastern province and one was conducted every 5 years. The first study was conducted in 1999 by el-Harith et al[14] and Borgio et al[15] conducted the last study in 2016. Four different studies on the prevalence of β-thalassemia were conducted in 20 years, which is very low for a region with such high prevalence (**Figures 1 & 2**). Various studies have reported that the Eastern Province of Saudi Arabia had the highest prevalence of both β-thalassemia and sickle cell disease (**Figures 1 & 2**).[2,16-20] However, according to the latest study on premarital screening and genetic counseling, which was performed in 2011, Jazzan, which is located in the southern region of Saudi Arabia, had the second highest prevalence of β-thalassemia in Saudi Arabia (**Figures 1 & 2**).[8] However, no studies about the molecular basis of the disease in the Jazzan region have been conducted. The prevalence of sickle cell disease carriers is more than double the prevalence of β-thalassemia carriers in all regions of Saudi Arabia (**Figure 1**). No studies on the molecular nature of the hemoglobinopathies have been conducted on a national level in Saudi Arabia. Hence, this review article is an attempt to list all the reported mutations and combine them in a single paper. This data can be used to facilitate further national-level studies to create a national repository of all *HBB* gene variations.

***Etiology.*** The HbVar database contains more than 800 variations of the β-globin gene, including consensus splice sites variations, nonsense codon variations, splice junction variations, frameshift variations, cryptic splice site variations, variations at 5'UTR, nonsense codon variations and point variations (**Table 1**).[21,22] Forty-two *HBB* gene variations were identified from various studies that were conducted either in Saudi Arabia or outside Saudi Arabia but with samples including Saudis.[12,14,16,17,23-28] In this review, the mutations are listed in **Table 2** in a specific order that shows how many studies reported the mutation. The Asian mutation IVSI-5 (G>C) tops the list, and the Mediterranean Cd39(C>T) mutation is second on the list (**Table 2**). The IVSI-5(G>C), Cd39(C>T), IVSII-1 (G>A), and IVSI-25bp mutations were reported in every prevalence study of β–thalassemia in Saudi Arabia (**Table 2**). The oldest record of a β-thalassemia mutation detected in a Saudi patient was from a study conducted abroad in

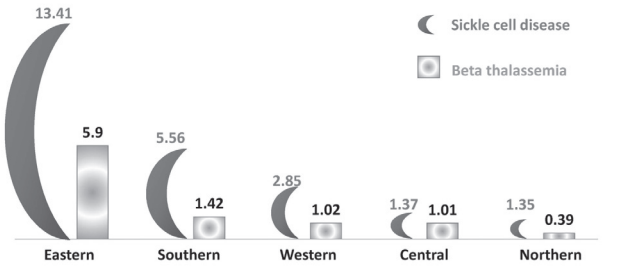


**Figure 1 -** Histogram shows the percentages of population who screened with HBB mutations either as homozygous or heterozygous (carrier/affected).

**Disclosure**. Authors have no conflict of interests, and the work was not supported or funded by any drug company.

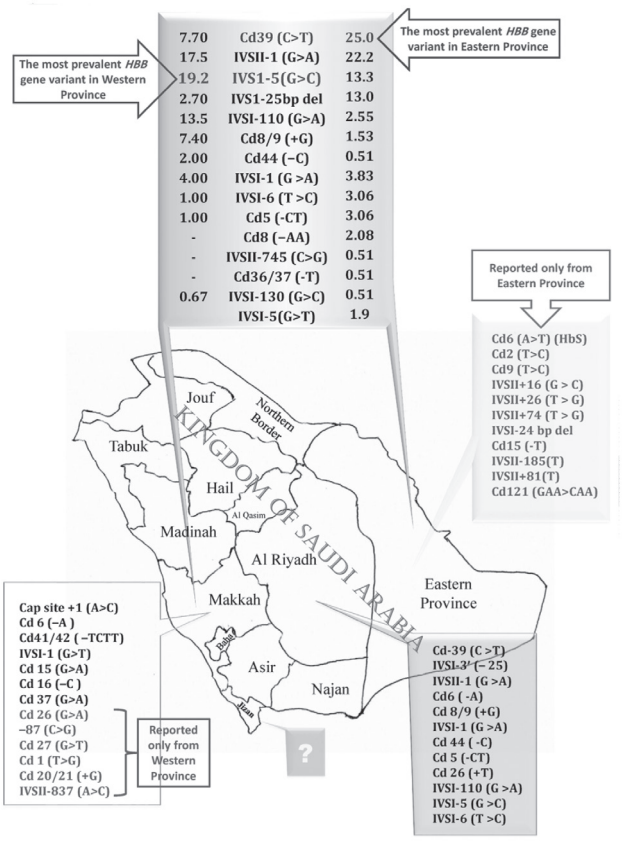


**Figure 2 -** Map illustrates the spectrum of various *HBB* gene variations. It turns out that the Eastern Province and Makkah region which were with more number of mutations through prevalence studies in Saudis. Hufuf, Qatif, and Dammam are the centers where the reported mutations came from at the studies in the Eastern Province.

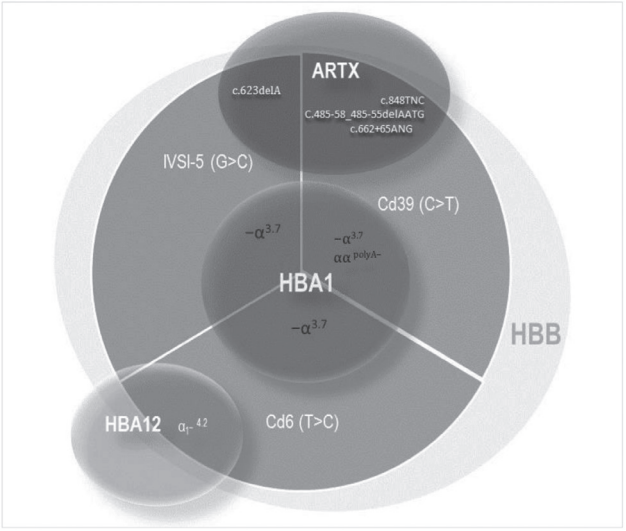


**Figure 3 -** Illustration for the co-inheritance of other gene mutations with *HBB* gene mutations. The outer grey circle imbedded with a tri-divided colored circle represented 3 *HBB* gene variations. Each one of the *HBB* mutation segment overlap exhibits the co-existence of other mutations that floated above them.

1986 by Boehm et al.[23] This study characterized the Cd37 (G>A) mutation in a Saudi family. The IVS1-128 (TAG>GAG) mutation was the second oldest mutation to be characterized and was found in a Saudi patient in a study performed by Wong et al.[24] El-Hazmi et al[12] then initiated the first prevalence study of β-thalassemia-related gene variations in Saudi Arabia by identifying 14 β-thalassemia-causing mutations that were reported from around the country. In addition to these studies, a few other non-prevalence studies reported a few *HBB* gene variations among Saudis.[25,28]

A study conducted in the Jeddah region reported the highest number of variations (28 variations) in a single study.[26] Also, a recent prevalence study performed by Borgio et al[15] reported 6 mutations that were observed for the first time in Saudi Arabia in addition to 3 novel mutations (**Tables 1 & 2**). The intronic variations *HBB*:c.315 + 26T>G [IIVS+26(T>G)], *HBB*: c.315 + 81C>T [IVSII+81(C>T)], and *HBB*:c93- 23_94del (25-bp deletion) were found only in Saudi populations.[2]

There were 24 $\beta^0$ mutations (complete lack of β-globin protein production), 9 $\beta^+$ mutations (reduced production of functional β-globin protein), 5 $\beta^i$ mutations (intronic mutations), and 2 $\beta^{++}$ mutations (silent) found in the Saudi population (**Table 1**). All of the different types of *HBB* gene variations were reported from Saudi Arabia (**Table 1**). Frameshift *HBB* gene sequence variations were the most prevalent mutations among the β-thalassemia mutations in the Saudi Arabian population. The second most prevalent type was splice junction mutations, which hit the invariants GT or AG with 5 mutations. Only 2 mutations originated from Saudi Arabia [Cd37 (G>A) and IVS1-128 (T>G)], whereas most of the existing variations in Saudis were of Mediterranean origin (13 variations).[29] Seven were from Asian India, 4 from the north neighbors Turkey and Kurdistan, and the rest originated from different parts of the world.

***Co-inheritance.*** The co-inheritance of other gene (*ATRX, HBA1, HBA12,* and *AHSP*) mutations with *HBB* gene mutations was reported in Saudis (**Figure 3**).[2,18,19,28] Recent studies have reported the co-inheritance of *HBB* gene variations with ATRX gene variations, such as c.485-58_485-55delAATG, c.662+65A>G, c.623delA, and c.848T>C. These 4 *ATRX* gene variations were reported to be novel and have been identified only in the Saudi Arabian

**Table 1 -** Nature of *HBB* gene variations identified in Saudis.

| Mutation | Origin | $\beta^0$ / $\beta^+$ / $\beta^i$ | Functional impact | Type of variation | Refrences |
|---|---|---|---|---|---|
| IVSI-5 (G>C) | Asian Indian | $\beta^0$ | RNA processing | Consensus splice sites | 12,13,14,16,17,19,26,27,28 |
| Cd39 (C>T) | Mediterranean | $\beta^0$ | RNA translation | Nonsense codon | 12,13,14,16,17,19,26,27 |
| IVSII-1 (G>A) | Mediterranean/US black | $\beta^0$ | RNA processing | Splice junction | 12,13,14,16,17,26,27 |
| IVSI-25 bp | Asian Indian / UAE | $\beta^0$ | RNA processing | Splice junction | 12,13,14,16,17,26,27 |
| Cd8/9 (+G) | Mediterranean | $\beta^0$ | RNA translation | Frameshift | 12,13,14,17,26,27 |
| IVSI-110 (G>A) | Mediterranean | $\beta^+$ | RNA processing | Cryptic splice site | 12,13,17,26,27 |
| IVSI-1 (G>A) | Mediterranean | $\beta^0$ | RNA processing | Splice junction | 13,16,17,26,27 |
| Cd44 ( –C) | Kurdish | $\beta^0$ | RNA translation | Frameshift | 14,16,17,26,27 |
| Cd5 (–CT) | Mediterranean | $\beta^0$ | RNA translation | Frameshift | 16,17,26,27,28 |
| Cd6 (–A) | Mediterranean | $\beta^0$ | RNA translation | Frameshift | 12,13,26,27 |
| IVSII-745 (C>G) | Mediterranean | $\beta^+$ | RNA processing | Cryptic splice site | 13,16,17,26 |
| IVSI-6 (T>C) | Mediterranean | $\beta^{++}$ | RNA processing | Consensus splice sites | 13,16,17,26 |
| Cd 8 (–AA ) | Turkish | $\beta^0$ | RNA translation | Frameshift | 14,17,26 |
| Cd 36/37 (–T) | Kurdish/Iranian | $\beta^0$ | RNA translation | Frameshift | 16,17,26 |
| Cap site +1 (A>C) | Asian Indian | $\beta^{++}$ | Transcription | 5'UTR | 12,13,26 |
| Cd 15 (TGG>TGA) | Portuguese/Japanese | $\beta^0$ | RNA translation | Nonsense codon | 13,25,26 |
| IVSI-5 ( G>T) | Mediterranean | $\beta^+$ | RNA processing | Consensus splice site | 14,26 |
| Cd 41/42,( –TCTT) | Asian Indian | $\beta^0$ | RNA translation | Frameshift | 13,26 |
| IVSI-1 (G>T) | Asian Indian/Chinese | $\beta^0$ | RNA Processing | Splice Junction | 14,26 |
| IVSI-130 (G>C) | Turkish | $\beta^0$ | RNA processing | Splice Junction | 17,26 |
| Cd37 (G>A) | Saudi | $\beta^0$ | RNA translation | Nonsense codon | 23,26 |
| Cd 16 (–C ) | Asian Indian | $\beta^0$ | RNA translation | Frameshift | 13,26 |
| Cd6 (A>T)(Hb S) | Mediterranean | $\beta^S$ | RNA translation | | 17,28 |
| Cd 26 (G>A) (Hb E) | South Asian | $\beta^+$ | RNA processing | Cryptic splice site | 26 |
| –87 (C>G) | Mediterranean | $\beta^+$ | Transcription | Promoter regulatory element | 26 |
| Cd27 (G>T) (Hb Knossos) | Mediterranean | $\beta^+$ | RNA processing | Cryptic splice site | 26 |
| Cd 1 (T>G) | Chinese | $\beta^0$ | RNA translation | Initiation codon | 26 |
| Cd 20/21 (+G) | Israeli | $\beta^0$ | | | 26 |
| IVSII-837 (A>C) | Asian Indian | $\beta^+$ | | | 26 |
| Cd26 (+T) | Japanese | $\beta^0$ | RNA translation | Frameshift | 27 |
| Cd1 (T>C) | Yugoslavian | $\beta^0$ | RNA translation | Initiation codon | 15 |
| Cd3 (T>C) | | $\beta^0$ | RNA translation | | 15 |
| IVSII+16 (G > C) | | $\beta^i$ | RNA processing | | 15 |
| IVSII+26 (T > G) | | $\beta^i$ | RNA processing | | 15 |
| IVSII+74 (T > G) | | $\beta^i$ | RNA processing | | 15 |
| IVSI-24 bp del | | $\beta^0$ | RNA processing | | 15 |
| Cd15 (-T) | Malay | $\beta^0$ | RNA translation | Frameshift | 15 |
| IVSII-185(C>T) | | $\beta^i$ | RNA processing | | 15 |
| IVSII+81(C>T) | | $\beta^i$ | RNA processing | | 15 |
| 619 bp del | Indian | $\beta^+$ | | | 28 |
| Cd121 (G>C) | HbD Los Angles | | RNA translation | Missense codon | 25 |
| IVS1-128 (T>G) | Saudi | $\beta^+$ | RNA processing | Consensus splice site | 24 |

$\beta^0$ - complete lack of β-globin proteins, $\beta^+$ - reduced production of functional β-globin protein, $\beta^i$ - intronic mutations, *HBB* - β-*hemoglobin gene*

population thus far.[19] Out of these 4, 2 were exonic (on exon 8 and exon 9) in nature, and the authors reported a family history of developmental disability. It would be more appropriate to conduct a national-level survey on the co-inheritance of other gene mutations with *HBB* gene mutations. Because of the high rate of consanguinity in the Saudi population, the inclusion of screening for mutations in the ATRX gene, followed by proper genetic counseling, may decrease the prevalence of offspring with inherited genetic disorders.Studies on the co-inheritance of *HBA1, HBA2*, and *HBA12* gene variations along with *HBB* gene variations have been

**Table 2 -** List of mutations reported in Saudi Arabia or in Saudis anywhere else.

| Mutation | NTR | HGVS Nomenclature | $\beta^0$ / $\beta^+$ / $\beta^i$ | 1999 KSA | 1999 East | 2005 East | 2011 East | 2011 West | 2012 Riyadh | References |
|---|---|---|---|---|---|---|---|---|---|---|
| IVSI-5 (G>C) | 9 | *HBB*:c.92+5G>C | $\beta^+$ | 12.9 | 1.9 | 23.2 | 13.3 | 19.2 | 24* | 4,12,14,16,17,26,27,28,19 |
| Cd39 (C>T) | | *HBB*:c.118C>T | $\beta^0$ | 12.9 | 32.1* | 20.3 | 25 | 7.7 | 12.8 | 4,12,14,16,17,26,27,19 |
| IVSII-1 (G>A) | 7 | *HBB*:c.315+1G>A | $\beta^0$ | 12.9 | 15.1 | 27.5* | 22.2 | 17.5 | 14.7 | 4,12,14,16,17,26,27 |
| IVSI-25 bp | | *HBB*:c.93-21_96del | $\beta^0$ | 12.9 | 22.6* | 4.4 | 13 | 19.2 | 11 | 4,12,14,16,17 |
| Cd8/9 (+G) | 6 | *HBB*:c.27_28insG | $\beta^0$ | 1.07 | 3.8 | | 1.53 | 7.4* | 0.92 | 4,12,14,17,26 |
| IVSI-110 (G>A) | | *HBB*:c.93-21G>A | $\beta^+$ | 26.9* | | | 2.55 | 13.5 | 24 | 4,12,17,26,27 |
| IVSI-1 (G>A) | 5 | *HBB*:c.92+1G>A | $\beta^0$ | | | 5.8 | 3.83 | 4 | 0.92 | 4,16,17,26,27 |
| Cd44 ( –C) | | *HBB*:c.135delC | $\beta^0$ | | 7.5 | 1.5 | 0.51 | 2 | 0.92 | 14,16,17,26,27 |
| Cd5 (–CT) | | *HBB*:c.17_18delCT | $\beta^0$ | | | 1.5 | 3.06 | 1 | 1.84 | 16,17,26,27,28 |
| Cd6 (–A) | | *HBB*:c.19delA | $\beta^0$ | 4.5 | | | | | 3.7 | 4,12,26,27 |
| IVSII-745 (C>G) | 4 | *HBB*:c.316-106C>G | $\beta^+$ | | | 1.5 | 0.51 | | | 12,16,17,26 |
| IVSI-6 (T>C) | | *HBB*:c.92+6T>C | $\beta^+$ | | | 4.4 | 7.14 | 1 | 0.92 | 12,16,17,26 |
| Cd 8 (–AA ) | | *HBB*:c.25_26delAA | $\beta^0$ | | 15.1 | | 2.08 | | | 14,17,26 |
| Cd 36/37 (–T) | 3 | *HBB*:c.112delT | $\beta^0$ | 1.07 | | 1.5 | 0.51 | | | 16,17,26 |
| Cap site +1 (A>C) | | *HBB*:c.-50A>C | $\beta^+$ | | | | | 1.4 | | 4,12,26 |
| Cd 15 (G>A) | | *HBB*:c.47G>A | $\beta^0$ | | 1.9 | | | 0.34 | | 12,25,26 |
| IVSI-5 ( G>T) | | *HBB*:c.92+5G>T | $\beta^+$ | | | | | | | 14,26 |
| Cd 41/42,( –TCTT) | | *HBB*:c.125_128delTCTT | $\beta^0$ | | | | | 2.4 | | 12,26 |
| IVSI-1 (G>T) | | *HBB*:c92+1G>T | $\beta^0$ | | | | | 1 | | 14,26 |
| IVSI-130 (G>C) | 2 | *HBB*:c.93-1G>C | $\beta^0$ | | | | 4.34 | 0.67 | | 17,26 |
| Cd37 (G>A) | | *HBB*:c.114G>A | $\beta^0$ | | | | | 2.4 | | 23,26 |
| Cd 16 (–C ) | | *HBB*:c.51delC | $\beta^0$ | | | | | 0.67 | | 14,26 |
| Cd6 (A>T)(Hb S) | | *HBB*:c.20A>T | $\beta^S$ | | | | | | | 17,28 |
| Cd 26 (G>A) (Hb E) | | *HBB*:c.79G>A | $\beta^E$ | | | | | 9.1 | | 26 |
| –87 (C>G) | | *HBB*:c.-137C>G | $\beta^+$ | | | | | 0.34 | | 26 |
| Cd27 (G>T) (Hb Knossos) | | *HBB*:c.82G>T | $\beta^+$ | | | | | 0.34 | | 26 |
| Cd 1 (T>G) | | *HBB*:c.2T>G | $\beta^0$ | | | | | 0.34 | | 26 |
| Cd 20/21 (+G) | | *HBB*:c.63_64insG | $\beta^0$ | | | | | 0.34 | | 26 |
| IVSII-837 (A>C) | | *HBB*:c.316-13C>A | $\beta^+$ | | | | | 0.67 | | 26 |
| Cd26 (+T) | | *HBB*:c.79_80insT | $\beta^0$ | | | | | | | 27 |
| Cd1 (T>C) | | *HBB*:c.2T>C | $\beta^0$ | | | | | | | 2 |
| Cd3 (T>C) | | *HBB*:c.9T>C | $\beta^0$ | | | | | | | 2 |
| IVSII+16 (G> C) | 1 | *HBB*:c.315+16G>C | $\beta^i$ | | | | | | | 2 |
| IVSII+26 (T > G) | | *HBB*:c.315+26T>C | $\beta^i$ | | | | | | | 2 |
| IVSII+74 (T > G) | | *HBB*:c.315+74T>C | $\beta^i$ | | | | | | | 2 |
| IVSI-24 bp del | | *HBB*:c.93-23_94del | $\beta^0$ | | | | | | | 2 |
| Cd15 (-T) | | *HBB*:c.46delT | $\beta^0$ | | | | | | | 2 |
| IVSII-185(C>T) | | *HBB*:c.316-185C>T | $\beta^i$ | | | | | | | 2 |
| IVSII+81(C>T) | | *HBB*:c.315+81C>T | $\beta^i$ | | | | | | | 2 |
| 619 bp del | | NG_000007.3:g.71609_72227del619 | $\beta^+$ | | | | | | | 28 |
| Cd121 (G>C) | | *HBB*:c.364G>C | - | | | | | | | 25 |
| IVS1-128 (T>G) | | *HBB*:c.93-3T>G | - | | | | | | | 24 |

The columns show all the recorded frequencies for *HBB* mutations within the study region in Saudi Arabia. *Region with highest prevalence of the particular mutation.
$\beta^0$ - complete lack of β-globin proteins, $\beta^+$ - reduced production of functional β-globin protein, $\beta^i$ - intronic mutations, $\beta^S$ - sickled,
*HBB* - β-*hemoglobin gene*, NTR - Number of times reported

performed in the Saudi population.[18,28] Akthar et al[18] reported the co-inheritance of 12 different α-globin gene variations in transfusion-dependent β-thalassemia major patients in the Eastern Province of the Kingdom of Saudi Arabia. In the healthy population, the -α3.7 deletion was reported to be the highest among the 12 different co-inherited α-globin gene variations in β-thalassemia major patients.[18] The co-inheritance of

different α-globin gene variations with the β-globin gene variations significantly reduced the levels of alanine transaminase and aspartate transaminase and the percentage incidence of osteoporosis, fracture, and splenectomy among the transfusion-dependent β-thalassemia major patients in the Eastern Province of the Kingdom of Saudi Arabia.[18]

Very recently, there was a report on the co-inheritance of *KLF1* gene variations [c.304T>C; c.544T>C; c.*296G>A; and c.*277C>G] with *HBB* gene variations (c.20A>T; c.25_26delAA; IVS I-5 (G→C); c.9T>C; c.17_18delCT; c.2T>C; NG_000007.3: g.71609_72227del619; c.46delT and c.93-23_94del) among β-thalassemia carriers from the Saudi Arabian population. The authors claimed that the 4 variations on the *KLF1* gene (c.304T>C; c.544T>C; c.*296G>A; and c.*277C>G) were not significantly associated with borderline *HbA2* among Saudi β-thalassemia carriers.[30] The co-inheritance of variations at *AHSP* genes (*AHSP*:c.167T>G; *AHSP*:c.45G>T; *AHSP*:c.231G>T; and *AHSP*:c.168G>T) with *HBB* gene variations was also reported in the Saudi population.[2]

***In conclusion and future research.*** β-hemoglobin gene variations, such as IVSI-5 (G>C) and Cd 39 (C>T), were the most prevalent among 42 variations that were reported in Saudis. The co-inheritance of *HBB* gene variations and variations in other genes, such as *ATRX, AHSP, HBA1, HBA2, HBA12*, and *KLF1* were observed to be common in Saudi Arabians with β-thalassemia. Studies on the prevalence of β-thalassemia in Saudi Arabia were centered in the Eastern Province due to the high prevalence of hemoglobinopathies in that area. However, compared to the number of world-wide studies, the number of extant studies is still considered insufficient. No molecular studies have been performed in the region of Jazzan, which has the second highest prevalence of β-thalassemia. The region of Makkah does not have a high prevalence of hemoglobinopathies, but, because it is the site of many research institutions in Saudi Arabia, many studies on β-thalassemia have been conducted there. This review suggests that an affected region, such as Jazzan, should have a molecular research laboratory for hemoglobinopathies. The actual reasons for the presence of borderline *HbA2* in Saudi β-thalassemia carriers remain unclear.[31] To identify all the pathogenic variants, DNA sequencing-based molecular diagnosis protocols should be included in the existing premarital screening program. No recent studies on the molecular basis of hemoglobin-related disorders have been conducted on a national level in Saudi Arabia. Therefore, it is highly recommended that Kingdom-based national surveys on the molecular nature of blood disorders be arranged through collaborations between research centers from various regions to create a well-documented molecular data bank of blood disorders. Such studies can reveal the effect of co-inheritance on hemoglobinopathies and lead to the development of the best treatment and preventive strategies for β-thalassemia. Furthermore, a very recent study on the level of *HbA2* and the presence of the *HBB* mutation proved that the level of *HbA2* is not a diagnostic parameter for the identification of β-thalassemia carriers.[31] Hence, the listed *HBB* variants can be identified in individuals through proper and simple technologies that minimize false positive and false negative results.

**Acknowledgment.** *Authors would like to thank the Dean, Institute for Research and Medical Consultations (IRMC), Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia for her continuous support and encouragement. The author MAA wishes to express his appreciation to the Dean, IRMC, for giving a chance to work as volunteer at the Department of Genetic Research, IRMC. Authors thank Mr. Ranilo. M. Tumbaga, Mr. Horace T. Pacifico, and Mrs. Jee E. Aquino for their timely support. Line drawing of the map of figure 2 was drawn by Dr. Sayed AbdulAzeez and Dr. J. Francis Borgio.*

## References

1. Quek L, Thein SL. Molecular therapies in β-thalassaemia. ***Br J Haematol*** 2007; 136: 353-365.
2. Borgio JF, Al-Madan MS, AbdulAzeez S. Mutation near the binding interfaces at α-hemoglobin stabilizing protein is highly pathogenic. ***Am J Transl Res*** 2016; 8: 4224.
3. Feng L, Gell DA, Zhou S, Gu L, Kong Y, Li J, et al. Molecular mechanism of AHSP-mediated stabilization of α-hemoglobin. ***Cell.*** 2004; 119: 629-640.
4. El-Hazmi MA, Warsy AS. Appraisal of sickle-cell and thalassaemia genes in Saudi Arabia. ***East Mediterr Health J*** 1999; 5: 1147-1153.
5. Memish ZA, Saeedi MY. Six-year outcome of the national premarital screening and genetic counseling program for sickle cell disease and β-thalassemia in Saudi Arabia. ***Ann Saudi Med*** 2011; 31: 229.
6. Ministry of Health. Premarital Screening. [Accessed 2017 June 15th] Available from: https://www.moh.gov.sa/en/healthawareness/beforemarriage/Pages/default.aspx.
7. Al-Abdulkareem AA, Ballal SG. Consanguineous marriage in an urban area of Saudi Arabia: rates and adverse health effects on the offspring. ***J Community Health*** 1998; 23: 75-83.
8. Memish ZA, Owaidah TM, Saeedi MY. Marked regional variations in the prevalence of sickle cell disease and β-thalassemia in Saudi Arabia: findings from the premarital screening and genetic counseling program. ***J Epidemiol Glob Health*** 2011; 1: 61-68.
9. Carley A. Anemia: when is it not iron deficiency? ***Pediatr Nurs*** 2003; 29: 205.
10. Al-Awamy BH. Thalassemia syndromes in Saudi Arabia. Meta-analysis of local studies. ***Saudi Med J*** 2000; 21: 8-17.

11. Mosca A, Paleari R, Ivaldi G, Galanello R, Giordano PC. The role of haemoglobin A2 testing in the diagnosis of thalassaemias and related haemoglobinopathies. ***J Clin Pathol*** 2009; 62: 13-17.
12. El-Hazmi MA, Al-Swailem AR, Warsy AS. Molecular Defects in Beta-Thalassaemia in the Population of Saudi Arabia. ***Hum Hered*** 1995; 45: 278-285.
13. El-Hazmi MA, Warsy AS, Al-Swailem AR. The frequency of 14 β-thalassemia mutations in the Arab populations. ***Hemoglobin*** 1995; 19: 353-360.
14. El-Harith EH, KÜhnau W, Schmidtke J, Stuhrmann M, Nasserallah Z, Al-shahri A. Identification and clinical presentation of β thalassaemia mutations in the eastern region of Saudi Arabia. ***J Med Genet*** 1999; 36: 936-937.
15. Borgio JF, AbdulAzeez S, Naserullah ZA, Al-Jarrash S, Al-Ali RA, Al-Madan MS, et al. Mutations in the β-globin gene from a Saudi population: an update. ***Int J Lab Hematol*** 2016; 38.
16. Al-Ali AK, Al-Ateeq S, Imamwerdi BW, Al-Sowayan S, Al-Madan M, Al-Muhanna F, et al. Molecular bases of β-thalassemia in the Eastern Province of Saudi Arabia. ***J Biomed Biotechnol*** 2005; 2005: 322-325.
17. Al-Sultan A, Phanasgaonkar S, Suliman A, Al-Baqushi M, Nasrullah Z, Al-Ali A. Spectrum of β-thalassemia mutations in the eastern province of Saudi Arabia. ***Hemoglobin*** 2011; 35: 125-134.
18. Akhtar MS, Qaw F, Borgio JF, Albuali W, Suliman A, Nasserullah Z, Al-Jarrash S, Al-Ali A. Spectrum of α-thalassemia mutations in transfusion-dependent β-thalassemia patients from the Eastern Province of Saudi Arabia. ***Hemoglobin*** 2013; 37: 65-73.
19. Al-Nafie AN, Borgio JF, AbdulAzeez S, Al-Suliman AM, Qaw FS, Naserullah ZA, et al. Co-inheritance of novel ATRX gene mutation and globin (α & β) gene mutations in transfusion dependent beta-thalassemia patients. ***Blood Cells Mol Dis*** 2015; 55: 27-29.
20. Borgio JF. Molecular nature of alpha-globin genes in the Saudi population. ***Saudi Med J*** 2015; 36: 1271.
21. Patrinos GP, Giardine B, Riemer C, Miller W, Chui DH, Anagnou NP, et al. Improvements in the HbVar database of human hemoglobin variants and thalassemia mutations for population and sequence variation studies. ***Nucleic Acids Res*** 2004; 32: 537-541.
22. Giardine B, van Baal S, Kaimakis P, Riemer C, Miller W, Samara M, et al. HbVar database of human hemoglobin variants and thalassemia mutations: 2007 update. ***Hum Mutat*** 2007; 28: 206.
23. Boehm CD, Dowling CE, Waber PG, Giardina PJ, Kazazian HJ. Use of oligonucleotide hybridization in the characterization of a beta zero-thalassemia gene (beta 37 TGG----TGA) in a Saudi Arabian family. ***Blood*** 1986; 67: 1185-1188.
24. Wong C, Antonarakis SE, Goff SC, Orkin SH, Forget BG, Nathan DG, et al. Beta-thalassemia due to two novel nucleotide substitutions in consensus acceptor splice sequences of the beta-globin gene. ***Blood***. 1989; 73: 914-918.
25. Ahmed M, Stuhrmann M, Bashawri L, Kühnau W, El-Harith EH. The β-globin genotype E121Q/W15X (cd121GAA→ CAA/cd15TGG→ TGA) underlines Hb D/β-(0) thalassaemia marked by domination of haemoglobin D. ***Ann Hematol*** 2001; 80: 629-633.
26. Abuzenadah AM, Hussein IM, Damanhouri GA, A-Sayes FM, Gari MA, Chaudhary AG, et al. Molecular basis of β-thalassemia in the western province of Saudi Arabia: Identification of rare β-thalassemia mutations. ***Hemoglobin*** 2011; 35: 346-357.
27. Warsy AS, El-Hazmi MA, Aleem A, Al-Hazmi AM, Al-Momin AK. Genetic basis of Saudi beta thalassemia identification of Mediterranean and Asian Mutations. ***Biosci Biotech Res Asia*** 2012; 9: 97-104.
28. Borgio JF, AbdulAzeez S, Al-Nafie AN, Naserullah ZA, Al-Jarrash S, Al-Madan MS, et al. A novel HBA2 gene conversion in cis or trans: "α12 allele" in a Saudi population. ***Blood Cells Mol Dis*** 2014; 53: 199-203.
29. Thein SL. The molecular basis of β-thalassemia. ***Cold Spring Harb Perspect Med*** 2013; 3: a011700.
30. Borgio JF, AbdulAzeez S, Al-Muslami AM, Naserullah ZA, Al-Jarrash S, Al-Suliman AM, et al. KLF1 gene and borderline hemoglobin A2 in Saudi population. ***Arch Med Sci*** 2018; 14: 230.
31. Al-Amodi AM, Ghanem NZ, Aldakeel SA, Ibrahim Al Asoom L, Rafique Ahmed N, Almandil NB, et al. Hemoglobin A2 (HbA2) has a measure of unreliability in diagnosing β-thalassemia trait (β-TT). ***Curr Med Res Opin*** 2018; 15: 1-7.