Identification of seven novel variants in the β-globin gene in transfusion-dependent and normal patients

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Abstract

Introduction: Abnormality in HBB results in an inherited recessive blood disorder, which can be caused by variants at the transcriptional or translational level affecting the stability and the production of the HBB chain. The severity of the disease relies on the variant’s characteristics. This study aimed to identify the common β-globin HBB variants in the population of the Eastern Province, which has the highest prevalence of blood diseases in Saudi Arabia.

Material and methods: Direct sequence of β-globin HBB gene, and alpha-globin HBA1 and HBA2 genes was performed on a total of 545 blood samples (transfusion-dependent: 215, 106 men and 109 women; normal healthy subjects: 330, 197 men and 133 women) collected from Saudi Arabian participants in the Eastern region.

Results: A total of 36 variants in HBB gene were revealed with 11 variants that have been reported for the first time in Saudi Arabia, including 7 novel variants that have been identified for the first time in HBB gene. The novel variants consisted of two exonic (HBB:c.252C>T; HBB:c.281G>T) and five intronic variants (c.316-183_316-168del; c.315+241T>A; c.315+376T>C; c.316-114C>G; c.315+208T>G) at HBB gene. The novel exonic variants and three (c.316-183_316-168del; c.315+241T>A; c.315+376T>C) intronic variants were co-inherited with α deletion.

Conclusions: This current study updated the HBB gene variations with newly identified variants of HBB gene and co-inheritance with α-globin deletions. The identified β-globin mutations will strengthen the genetic reference that could aid in characterizing mutations that are associated with phenotype of thalassemia in a specific region.

Key words: HBB gene, novel variants, hemoglobin, hematological disorders, DNA sequencing.

Introduction

β-Globin protein (HBB), one of the hemoglobin subunits, is produced by β-globin gene (HBB), which is located on chromosome 11 [1]. Two β-globin
molecules bind to two α-globin molecules to consti-
tute the most popular form of hemoglobin, adult he-
moglobin (HbA). HBB is crucial in balancing the ratio
of (α : β)-globin chains, preventing the aggregation of
insoluble α-globin complex [1–3]. Abnormality in the
HBB gene results in an inherited recessive blood dis-
order that can be caused by variants at the transcrip-
tion or translation level affecting the stability and the
production of the β-globin chain [4, 5].

Several HBB variants are produced by mutations in
HBB gene, and some mutations alters the production
of HBB chain either partially (β+) or completely
(β–) [6]. Reduced formation of HBB chain lowers the
amount of functional Hb, which is a characteristic of
the highly prevalent blood disorder in Saudi Arabia,
β-thalassemia [7–12]. Variations in HBB protein can
also be associated with other genetic hematological
disorders such as sickle cell disease, which is very
common in Saudi Arabia. To date, there are more
than 1,700 hemoglobin variants that have been
reported, with more than 900 variants in HBB [13];
however, the most common variants are hemoglo-
in E (HbE), sickle hemoglobin (HbS), and hemoglo-
in C (HbcC) that results as a consequence of point
mutations in HBB gene [14]. The formation of the
highly unstable α and β complex HbE is a result of
a point mutation at position 26 in HBB that substi-
tuted glutamic acid with lysine, causing a pheno-
typic characteristic of a mild form of β-thalassemia
[15].

During the production of HbS, the most common
cause of sickle cell disease is a consequence of a
point mutation at the sixth position in HBB gene
that substituted glutamic acid codon (GAG) with
valine codon (GTG) [16]. Additionally, glutamic acid
can be substituted with lysine forming HbcC, which is
a Hb variant that is related to sickle cell disease [5].

On the other hand, some variants might have ben-
eficial influences. For instance, it has been known
that individuals with HbcC are protected at different
effective influences. For instance, it has been known
that individuals with HbcC are protected at different

Hematological parameters
Hematological parameters were evaluated
following the collection of blood samples (5 ml)
in EDTA-coated vacutainers using Coulter Micro
Diff II (Beckman Coulter, Inc., Brea, CA, USA) and
VARIANT™ II Hemoglobin Testing System (BIO-Rad
Laboratories, Inc., Hercules, CA, USA).

DNA extraction and sequencing
DNA was extracted from all of the 545 blood
samples utilizing QIAamp DNA blood minikit (Qia-
gen, GmbH, Hilden, Germany) followed by amplifi-
cation of HBB, HBA1 and HBA2 genes using the
PCR standard method [8, 20–21]. Utilizing forward
and reverse primers separately, HBB, HBA1, and
HBA2 genes in each sample were amplified by
PCR using BigDye Terminator Cycle Sequencing
Kit (Thermo Fisher Scientific, Inc., Waltham, MA,
USA). Following the amplification of each gene,
purification was conducted to prepare the sam-
ple for sequencing, which was performed using the
series Genetic Analyzer 3500 (Thermo Fisher
Scientific, Inc.) at the Department of Genetic Re-
search, Institute for Research and Medical Consul-
tation, Imam Abdulrahman Bin Faisal University
(Dammam, Saudi Arabia). DNA sequencing analy-
sis software v. 5.3 (Applied Biosystem; Thermo
Fisher Scientific, Inc.) and mutation surveyor soft-
ware (Softgenetics, US) were used to analyze elec-
tropherograms.

Results
Conducting a direct sequence of HBB gene
from 545 (215 transfusion-dependent subjects
and 330 normal healthy subjects) subjects re-
vealed that the 249 subjects were with nor-
mal HBB gene and 296 subjects were identified

Material and methods
Samples collection
A total of 545 blood samples were collected
from male (n = 303) and female (n = 242) par-
ticipants from the Eastern region of Saudi Arabia.
This study included 215 transfusion-dependent
subjects (age range: 2 months to 51 years; 106
males and 109 females) and 330 normal healthy
subjects (age range: 8 months to 67 years; 197
males and 133 females), who attended major
hospitals in the region. Samples were requested
from random volunteers and patients who were
clinically diagnosed with β/α-thalassemia major/
carriers or sickle cell anemia. Transfusion-depen-
dent subjects include 20 sickle cell disease (ho-
mozygous) patients and 195 β-thalassemia major
patients. Subjects with no history of blood trans-
fusion, blood disorders, or chronic diseases were
included as normal control.

The study was approved by the Standing
Committee for Research Ethics on Living Crea-
tures, Imam Abdulrahman Bin Faisal University
(CBME2012032; IRB-2015-08-069).

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Results
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with 36 (HBB:c.118C>T; HBB:c.315+1G>A; HBB:c.93-21_96del; HBB:c.20A>T; HBB:c.92+5G>C; HBB:c.92+6T>C; HBB:c.17-18delCT; HBB:c.93-21G>A; HBB:c.25_26delAA; HBB:c.97C>T; HBB:c.27_28insG; HBB:c.79G>A; HBB:c.92+1G>A; HBB:c.431T>A; HBB:c.27T>C; HBB:c.112delT; HBB:c.315+74T>G; HBB:c.415G>A; HBB:c.432C>T; HBB:c.431T>A; HBB:c.2T>C; HBB:c.218G>C; HBB:c.253A>G; HBB:c.294C>T; HBB:c.135delC; HBB:c.93-1G>C; HBB:c.370A>C; HBB:c.320T>C; HBB:c.315+208T>G; HBB:c.315+241T>A; HBB:c.315+376T>C; HBB:c.316-183_316-168del; HBB:c.252C>T, and HBB:c.316-114C>G) different types (β+: a complete lack of β-globin production; β0: a partial production of β-globin; β−: β-intercranial mutation; whereas βi is HbE disease) of variants in the exonic and intronic regions of HBB gene. Among the 36 HBB variants, 25 variants have been previously reported from the Saudi Arabian population, the remaining 11 variants have been identified for the first time in this study in Saudi Arabians. Among these 11 variants, 7 (2 exonic and 5 intronic) variants have been identified for the first time in the HBB gene in the study subjects. These 7 variants (HBB:c.281G>T; HBB:c.316-183_316-168del; HBB:c.315+208T>G; HBB:c.315+241T>A; HBB:c.252C>T, and HBB:c.316-114C>G) have not been reported earlier in any population; hence, these 7 variants were described in detail in Table I and are referred as novel variants.

Twenty-six HBB variants (HBB:c.118C>T; HBB:c.20A>T; HBB:c.315+1G>A; HBB:c.93-21_96del; HBB:c.92+5G>C; HBB:c.92-21G>A; HBB:c.17-18delCT; HBB:c.25_26delAA; HBB:c.27_28insG; HBB:c.79G>A; HBB:c.431T>A; HBB:c.27T>C; HBB:c.112delT; HBB:c.315+74T>G; HBB:c.415G>A; HBB:c.432C>T; HBB:c.316-183_316-168del; HBB:c.252C>T, and HBB:c.316-114C>G) are variants that present in the non-coding regions of HBB gene (Figure 1). These novel HBB variants in normal healthy group are co-inherited with α-globin deletion.

Furthermore, in an effort to test the notion that the presence of these seven novel identified variants would have a more influence on red blood cell pathophysiology compared to control/sickle cell/thalassemia conditions, the general hematological parameters of blood samples were evaluated. In each sample, the co-inheritance of the novel mutation/mutations at the intronic regions seems to demonstrate no influence on blood parameters compared with existing of these conditions alone. However, one of the normal control subjects had a novel exonic missense mutation: HBB:c.281G>T and borderline HbA2 level, i.e. 3% (Table I).

Two males and 3 females transfusion-dependent subjects had a total of four novel variants; 3 point mutations; HBB:c.252C>T, HBB:c.281G>T, and HBB:c.316-114C>G as well as one deletion; HBB:c.316-183_316-168del (Figure 1, Table I). The HBB:c.252C>T is a silent point mutation, where the change in the codon results on encoding for the same amino acid, glycine. The point mutation; HBB:c.281G>T is a missense mutation, which results on encoding for the amino acid phenylalanine rather than cysteine. The point mutation, HBB:c.316-114C>G is present in the intronic region of HBB gene. The newly reported deletion (HBB:c.316-183_316-168del) was observed in two transfusion-dependent patients who are clinically diagnosed with sickle cell anemia. The most common α-thalassemia mutation amongst these transfusion-dependent subjects was the 3.1 single gene deletion. However, one of the subjects had HBA1 intronic mutation (Table I).

On the other hand, four newly identified point mutations have been observed in normal control participants (2 males, 1 female): HBB:c.281G>T, HBB:c.315+208T>G; HBB:c.315+241T>A, and HBB:c.315+376T>C (Figure 1, Table I). HBB:c.281G>T is a point mutation that was identified also in a normal control subject, which results on the conversion of an UGU codon into UUU, which encodes the amino acid phenylalanine rather than cysteine. The remaining three point mutations: HBB:c.315+208T>G, HBB:c.315+241T>A, and HBB:c.315+376T>C are variants that present in the non-coding regions of HBB gene (Figure 1). These novel HBB variants in normal healthy group are co-inherited with α-globin deletion.

Discussion

HBB and HBA genes encode the normal adult hemoglobin tetramer (Hb), constituting of four polypeptide chains; 2 α chains and 2 β chains [23]. Mutations such as frameshift, minor deletions, and missense mutations in the HBB gene may result in highly unstable HBB protein [6, 24]. Various novel mutations have been still observed in HBB gene. Recently, Ekwattanakit et al.
Table I. Hematological features and genetic results of subjects with newly identified HBB mutations

| Parameter                        | Transfusion dependent novel mutation | Normal novel mutation |
|----------------------------------|--------------------------------------|----------------------|
|                                  | HBB:c.252C>T                         | HBB:c.281G>T         |
|                                  | HBB:c.316-183_316-168del             | HBB:c.315+1G>A       |
|                                  | HBB:c.316-114C>G                     | HBB:c.315+208T>A     |
|                                  | HBB:c.315+241T>A                     | HBB:c.315+376T>C     |
| N                                | 1                                    | 1                    |
| Region of mutation               | Exonic                               | Exonic               |
|                                  | Intrinsic                            | Intrinsic            |
| Mutation type                    | Silent mutation                      | Missense mutation    |
|                                  | Deletion                             | Point mutation       |
|                                  | HBB reported mutations               | Point mutation       |
|                                  | HBB:c.118C>T; HBB:c.315+1G>A;       | HBB:c.20A>T          |
|                                  | HBB:c.253A>G; HBB:c.26A>T           | HBB:c.20A>T          |
|                                  | HBB:c.315+1G>A; HBB:c.20A>T         |                      |
|                                  | HBB:c.316-114C>G                     |                      |
|                                  | HBB:c.315+208T>G                     |                      |
|                                  | HBB:c.315+241T>A                     |                      |
|                                  | HBB:c.315+376T>C                     |                      |
| Age                              | 9                                    | 9                    |
| Gender                           | M                                    | M                    |
| Hb [g/dl]                        | 8.6                                  | 10.3                 |
| Hbf [g/dl]                       | 8.3248                               | 10.0425              |
| Hbs (%)                          | –                                    | 45                   |
| HbaA (%)                         | 3.2                                  | 2.5                  |
| MCV [Fl]                         | 77                                   | 79                   |
| α-Globin genotype                | \(-α_{2}^{+}\)/α\_2\textsuperscript{-} | \(-α_{2}^{+}\)/α\_2, α\_2\textsuperscript{α} |
|                                  | \(-α_{2}^{+}\)/α\_2, α\_2\textsuperscript{α} | \(-α_{2}^{+}\)/α\_2, α\_2\textsuperscript{α} |
| Haematological disease           | Major \(β\)-thalassemic              | Major \(β\)-thalassemic |
|                                  | Sickle cell anaemia                  | Sickle cell anaemia  |
|                                  | HbH disease                          | – \(β\)-Thalassemic carrier |
| Haematological disease           | Major \(β\)-thalassemic              | Major \(β\)-thalassemic |
|                                  | Sickle cell anaemia                  | Sickle cell anaemia  |
|                                  | HbH disease                          | – \(β\)-Thalassemic carrier |
|                                  | –                                    | –                    |
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[25] have identified two frameshift mutations in the third exon that causes a rapid mRNA decay in thalassemia intermedia patients. The novel heterozygous mutation identified in this present study, HBB:c.281G>T in exon 2 is caused by a missense mutation that substituted the sulfur containing amino acid, cysteine into the aromatic amino acid, phenylalanine [26]. This substitution might alter the protein structure or interfere with the heme-binding pocket as it has been shown that sulfur containing amino acids form stronger interactions compared to the aromatic amino acids. This substitution might reduce the stability of the protein function [26]. Further studies on these novel mutations are needed to investigate any alteration in the proper function of HBB protein.

HBB produces an important component in building up hemoglobin complex, which carries on oxygen molecules into the cells. The production of β-globin chain affects the ratio of α and β, and in turns the production of an intact hemoglobin complex. In addition, the imbalance of α/β chains causes the formation of toxic complexes of free α chains aggregation [2, 27]. There are several genetics hematological disorders associated with genetic changes in the HBB. This genetic change might yield unfunctional, structurally abnormal β-chain variants, or affect the proteins’ production [4, 5]. However, it has been previously found that the co-inheritance of β-mutations with α-mutations might reduce the amount of α-globin production, which in turn reduce the imbalance of β/α chain resulting in the amelioration of disease severity [28].

It is worth to note that these novel mutations that have been identified in this study are co-inherited with other mutations, which are present in HBB gene or other genes that might directly interact with HBB. Therefore, it cannot be concluded whether the changes in the hematological parameters are influenced by the novel identified variants or as a result of other factors. To illustrate, the novel hetero-exonic mutation HBB:c.281G>T, which has one amino acid change from cysteine to phenylalanine, has been found solely in a β-thalassemia carrier as well as in a β-thalassemia major with other mutations. The β-thalassemia carrier subject had a borderline HbA2 level 3% (Table I), which could be a consequence of either the mutation or the co-inheritance of α deletion [3, 7] as it has been previously shown to increase HbA2 level [27, 29]. The severity of the phenotype in the β-thalassemia major subject might be affected by the presence of other mutations.

In an effort to understand the implications of these novel identified variants, number of future studies needs to be conducted. HBB gene consists of three exons and based on previous studies, the severity of the disease lies on which exon the mutations are located. For example, based on Cao and Galanello [6], the mutation in phase termination codon stimulates the nonsense-mediated mRNA decay (NMD) process, resulting
in low production of normal HBB chain [6, 24]. Since the two exonic novel mutations presented in this study are located in exon 2, further studies are required to investigate whether they have similar effects. Similarly, majority of the identified mutations in this study were present in intronic region, which can alter the gene’s function and expression in different ways, as non-coding regions play a role in transcriptional and translational regulation. Non-coding mutations may also introduce novel splice sites that result in a truncated protein, leading in loss of function [30]. In addition, the effect of homoygosity of these mutations is not distinguished. Thus, future studies could overcome this limitation by investigating the effect of inheriting two mutant alleles of these novel variants on the severity of the phenotype.

In conclusion, HBB molecular scanning for both transfusion-dependent and normal control subjects is an important step for better understanding and accurate diagnosis of hemoglobinopathies. This current study has identified 11 variants that have been reported for the first time in Saudi Arabia. Seven novel (2 exonic and 5 intronic) variants in HBB gene have been observed in the study population. The influence of these novel variants on the phenotypic characteristics and β-globin proper function is currently unknown and thus needs to be examined. The identified β-globin mutations could be used as a genetic to the study region.

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Conflict of interest

The authors declare no conflict of interest.

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