Alpha-globin gene triplication and its effect in beta-thalassemia carrier, sickle cell trait, and healthy individual

Mohammad Hamid1 | Bijan keikhaei2 | Hamid Galehdari3 | Alihossein Saberi4 | Alireza Sedaghat5 | Gholamreza Shariati4,6 | Marziye Mohammadi-Anaei6

1 Department of Molecular Medicine, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran
2 Research Center for Thalassemia and Hemoglobinopathy, Health Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
3 Department of Genetics, Faculty of Sciences, Shahid Chamran University of Ahvaz, Ahvaz, Iran
4 Department of Medical Genetics, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
5 Department of Endocrinology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
6 Narges Medical Genetics and PND Laboratory, Ahvaz, Iran

Abstract

The genotype and phenotype correlation between coinheritance of heterozygous beta-thalassemia with the alpha-globin triplication is unclear. In this study we have investigated and reviewed alpha triplication frequency in beta-thalassemia carriers, sickle cell trait, and healthy individuals and its effect on hematological and phenotypical changes. In this study, 4005 beta-thalassemia carriers, 455 sickle cell trait, and 2000 healthy individuals were included. Molecular characterization of beta and alpha-thalassemia was performed. The frequencies of alpha-globin triplication in beta-thalassemia carriers, sickle cell trait, and healthy individuals were 67 (1.67%), 4 (0.88%), and 18 (0.9%), respectively. In total, the frequency of alpha-triplications is approximately 89 (1.39%) in Khuzestan province, South of Iran population. We have compared the average hematological parameters of beta-thalassemia carriers, sickle cell trait, and healthy individuals with and without alpha gene triplication. This mutation did not show any significant effect on the change of blood indices, neither in healthy individuals nor in sickle cell trait and beta-thalassemia carriers. Therefore, there is no need to take more notice of anti 3.7 mutation in beta-thalassemia carriers is opposed with some studies reported that the presence of excess alpha-globin genes in beta-thalassemia carriers can lead to the phenotype of beta-thalassemia intermedia. Therefore, not every individual with triplicated alpha globin coinherited with beta-thalassemia trait will have a significantly lower Hb than normal, and it is highly likely that none of them will need transfusion.

KEYWORDS
alpha-globin triplication, beta-thalassemia, Iran

1 | INTRODUCTION

Between the major, transfusion-dependent forms of the disease and the symptomless carrier states is a thalassemia intermedia which is a milder form of the disease needing fewer or no transfusion and consequently less or no iron chelation. Generally, the level of hemoglobin in those who are affected by intermediate thalassemia is below 9–10 g/dl [1]. Beta-thalassemia is further classified into severe (hemoglobin level as low as 4–5 g/dl, transfusion-dependent, clinical symptoms similar to β-thalassemia major), moderate (hemoglobin levels between
Hematological parameters

2 PATIENTS AND METHODS

2.1 Ethical statement

This study was approved by the Ethics Review Committee of Pasteur Institute of Iran. Informed consent was signed and obtained from all participants following a detailed description of the purpose of the study. All methods were carried out in accordance with relevant guidelines and regulations.

2.2 Study subjects

The peripheral blood was taken from those referred to Narges Genetics Laboratory in Ahvaz city including 4005 beta-thalassemia carriers, 455 sickle cell trait, and 2000 healthy individuals from Khuzestan province, south of Iran. Hematological indices were automatically measured on a Coulter Counter ABX Micro 60 (Helena Laboratories, Beaumont, TX, USA). Hemoglobin electrophoresis was performed on cellulose acetate (Helena Laboratories) using Tris-EDTA-borate buffer (pH 8.4). The Khuzestan Province is located southwest of Iran with a population of about 4.7 million people based on the 2016 census with different ethnicities (Arab, Lur, Bakhtiaris, and Fars). In total, 89 alpha-triplication mutations have been detected among 6460 individuals tested. Therefore, in total the frequency of α-triplications is approximately 1.39% in Khuzestan province, south of Iran population.

2.3 Molecular studies

Molecular studies were conducted on genomic DNA isolated from peripheral blood cells by a salting-out procedure [7]. For identifying α-thalassemia genotype, investigation of common Mediterranean α-globin gene deletions (α3.7, α4.2, -α20.5 and -MED) and α3.7 triplication were performed [8]; the entire α- and β-globin genes were amplified and DNA sequenced, ABI -3130 (Applied Biosystems, Foster City, CA, USA) [9]. In some cases, multiplex ligation-dependent probe amplification (MLPA assay) was performed using the SALSA MLPA kit P140-B4 HBA (MRC-Holland, Amsterdam, Netherlands).

2.4 Data analysis

The results were examined using t-test, two tails and were compared on the p-value < 0.05 significance level.

3 RESULTS

3.1 Frequency of alpha-globin gene triplication

In this study, 4005 beta-thalassemia carriers, 455 sickle cell trait, and 2000 healthy individuals were chosen. The frequencies of alpha-globin gene triplication in three studied groups including beta-thalassemia carriers, sickle cell trait, and healthy individuals were 67 (1.67%), 4 (0.88%), and 18 (0.9%) respectively. So, it could be said that about 0.9% of the normal population, 1.67% of the beta-thalassemia carriers, and 0.88% sickle cell trait have anti 3.7 mutation in the south of Iran with different ethnicities (Arab, Lur, and Fars). In total, 89 alpha-triplication mutations have been detected among 6460 individuals tested. Therefore, in total the frequency of α-triplications is approximately 1.39% in Khuzestan province, south of Iran population.

3.2 Hematological parameters

The average hematological parameters of every studied genotype with alpha gene triplication in three groups were compared with the same mutations with the normal of the alpha-globin gene. Alpha gene triplication did not show any significant role in the changing of blood indices, in healthy individuals, sickle cell trait, and beta-thalassemia carriers. All beta-thalassemia heterozygotes with triplicated α-globin genes, were clinically asymptomatic, and none of them needed a blood transfusion. The data are summarized in Table 1. In this study the level of total hemoglobin in thalassemia intermedia is considered between 6 and 9 g/dl; hence, most of the beta-thalassemia carriers with 19 different mutations associated with alpha triplication were higher than 9 g/dl of hemoglobin. We have just had four beta-thalassemia carriers with alpha triplication (4 of 66, 6%) lower than 9 g/dl of hemoglobin (one female CD36/37-T/wt, 7.9 g/dl, one male IVSII-I (G-A)/ wt, 8.6 g/dl, one female CD8(-AA)/ wt, 8.5 g/dl, and one female CD8(-AA)/ wt, 8.6 g/dl) which none of them received blood transfusion.

4 DISCUSSION

There are different studies which showed the frequency of the alpha-globin gene triplication in healthy individuals and thalassemia patients.
| \( \beta \) genotype | \( \alpha \) genotype | \( n \) | Gender | Age mean ± SD | MCV (fL) mean ± SD | MCH (pg) mean ± SD | Hb (g/dl) mean ± SD | RBC (10¹²/L) mean ± SD | Hb A (%) mean ± SD | Hb A2 (%) mean ± SD | Hb F (%) mean ± SD |
|---------------------------|-----------------|------|--------|---------------|------------------|----------------|------------------|------------------|------------------|------------------|------------------|
| CD36/37-T/wt              | \( \alpha / \alpha \) | 265  | 137/128 | 24.7 ± 5.2    | 62.15 ± 3.36   | 19.03 ± 1.2    | 11.36 ± 1.3    | 6.08 ± 0.90      | 93.73 ± 1.24    | 5.25 ± 0.87      | 0.84 ± 0.73      |
| CD36/37-T/wt              | \( \alpha / \alpha \) | 17   | 9/8     | 27 ± 9.4      | 59.64 ± 4.8    | 18.45 ± 1.03   | 10.88 ± 1.21   | 5.6 ± 0.74       | 94.55 ± 0.40    | 5.07 ± 0.77      | 0.47 ± 0.22      |
| IVSII-(G-A)/wt            | \( \alpha / \alpha \) | 239  | 135/104 | 25.65 ± 7.0   | 63.32 ± 3.56   | 19.52 ± 1.36   | 11.37 ± 1.46   | 5.84 ± 0.82      | 94.02 ± 1.30    | 4.71 ± 0.95      | 1.46 ± 1.02      |
| IVSII-(G-A)/wt            | \( \alpha / \alpha \) | 10   | 5/5     | 27.7 ± 4.41   | 61.05 ± 2.01   | 19.58 ± 1.48   | 10.98 ± 1.56   | 5.54 ± 0.75      | 93.77 ± 3.05    | 4.97 ± 0.37      | 0.9             |
| CD44(-C)/wt               | \( \alpha / \alpha \) | 112  | 50/62   | 247 ± 3.2     | 62.79 ± 2.85   | 19.17 ± 0.79   | 11.46 ± 1.42   | 6.00 ± 0.66      | 94.38 ± 0.62    | 4.92 ± 0.53      | 0.57 ± 0.23      |
| CD44(-C)/wt               | \( \alpha / \alpha \) | 5    | 2/3     | 30.3 ± 3.3    | 58.50 ± 0.71   | 18.00 ± 2.82   | 10.40 ± 0.14   | 5.90 ± 0.28      | 94.45 ± 0.21    | 5.40 ± 0.28      |                  |
| 5’UTR+20(C-T)/wt          | \( \alpha / \alpha \) | 160  | 52/108  | 27.1 ± 3.35   | 61.79 ± 3.10   | 18.67 ± 0.87   | 12.05 ± 0.91   | 6.46 ± 0.57      | 94.15 ± 0.77    | 5.26 ± 0.71      | 0.52 ± 0.25      |
| 5’UTR+20(C-T)/wt          | \( \alpha / \alpha \) | 5    | 2/3     | 24.0 ± 5.5    | 66.50 ± 0.28   | 18.40 ± 0.56   | 11.3 ± 0.141   | 6.03 ± 0.05      | 93.45 ± 0.64    | 5.15 ± 0.21      |                  |
| CD82-83(G)/wt             | \( \alpha / \alpha \) | 123  | 62/61   | 290 ± 8.1     | 62.92 ± 2.9    | 19.66 ± 2.15   | 11.29 ± 1.52   | 5.78 ± 1.04      | 93.74 ± 1.16    | 5.00 ± 0.66      | 1.12 ± 0.82      |
| CD82-83(G)/wt             | \( \alpha / \alpha \) | 4    | 2/2     | 260 ± 8.5     | 61.00 ± 0.14   | 18.95 ± 0.63   | 11.7 ± 1.70    | ?                | ?                | ?                |                  |
| CD88(AA)/wt               | \( \alpha / \alpha \) | 175  | 120/55  | 294 ± 8.8     | 64.06 ± 2.3    | 19.87 ± 0.95   | 10.40 ± 0.95   | 5.37 ± 0.59      | 93.73 ± 0.59    | 4.98 ± 0.77      | 1.14 ± 0.66      |
| CD88(AA)/wt               | \( \alpha / \alpha \) | 3    | 3/0     | 222 ± 5.1     | 62.45 ± 0.77   | 20.80 ± 0.28   | 10.2 ± 1.13    | 4.68 ± 0.17      | 94.5 ± 0.70     | 4.85 ± 0.82      |                  |
| –28(A-C)/wt               | \( \alpha / \alpha \) | 125  | 62/63   | 254 ± 8.7     | 70.73 ± 3.02   | 22.33 ± 1.18   | 12.72 ± 1.08   | 5.71 ± 0.4       | 94.27 ± 0.57    | 4.91 ± 0.76      | 0.67 ± 0.32      |
| –28(A-C)/wt               | \( \alpha / \alpha \) | 2    | 1/1     | 23 ± 0.7      | 72.85 ± 0.63   | 22.7 ± 1.41    | 12.50 ± 0.70   | 4.8 ± 0.28       | ?                | ?                |                  |
| IVSII-6(T-G)/wt           | \( \alpha / \alpha \) | 126  | 59/67   | 27.14 ± 6.8   | 70.77 ± 4.71   | 21.83 ± 1.79   | 12.68 ± 1.55   | 5.81 ± 0.55      | 95.99 ± 0.73    | 3.58 ± 0.74      | 0.61 ± 0.31      |
| IVSII-6(T-G)/wt           | \( \alpha / \alpha \) | 2    | 1/1     | 255 ± 21      | 72.20 ± 0.28   | 22.6 ± 0.42    | 12.0 ± 0.3     | 5.24 ± 0.19      | 95.30 ± 0.14    | 3.85 ± 0.07      | 0.60 ± 0.14      |
| Fr8-9(+G)/wt              | \( \alpha / \alpha \) | 127  | 61/66   | 260 ± 5.2     | 62.82 ± 2.98   | 19.13 ± 1.33   | 11.36 ± 1.28   | 5.97 ± 0.69      | 94.09 ± 0.96    | 5.13 ± 0.86      | 0.8 ± 0.53       |
| Fr8-9(+G)/wt              | \( \alpha / \alpha \) | 3    | 1/2     | 235 ± 2.1     | 59.0 ± 1.41    | 18.5 ± 0.71    | 10.25 ± 1.06   | 5.50 ± 0.71      | 94.0 ± 0.42     | 5.5 ± 0.42       | 0.5 ± 0.0        |

(Continues)
| \( \beta \) genotype | \( \alpha \) genotype | \( n \) | Gender | Age mean ± SD | MCV (fL) mean ± SD | MCH (pg) mean ± SD | Hb (g/dl) mean ± SD | RBC (10^{12}/L) mean ± SD | Hb A (%) mean ± SD | Hb A2 (%) Mean ± SD | Hb F (%) Mean ± SD |
|---------------------|---------------------|------|--------|----------------|-----------------|-----------------|-----------------|-----------------|----------------|----------------|----------------|
| CD15(TGG-GA)/wt     | \( \alpha / \alpha \) | 125  | 60/65  | 26 ± 5.7 | 62.67 ± 2.30 | 19.25 ± 0.95 | 12.07 ± 1.31 | 63.3 ± 0.73 | 94.03 ± 0.68 | 5.05 ± 0.97 | 0.62 ± 0.42 |
| p-value             | 0.291               | 0.255| 0.245  | 0.422       | 0.724           | 0.890           | 0.579           |
| Indian deletion/wt  | \( \alpha / \alpha \) | 10   | 5/5    | 29 ± 7.7  | 58.94 ± 2.60 | 18.72 ± 1.23 | 10.97 ± 2.17 | 58.67 ± 1.09 | 96.53 ± 1.34 | 2.92 ± 0.64 | 0.66 ± 0.47 |
| p-value             | 0.727               | 0.290| 0.480  | 0.486       | 0.20            | 0.467           | 0.064           |
| −101(C > T)/wt      | \( \alpha / \alpha \) | 64   | 34/30  | 26 ± 5.4  | 79.48 ± 4.5   | 26.2 ± 1.9    | 14.0 ± 1.7   | 53.6 ± 0.64 | 95.82 ± 0.9  | 3.4 ± 0.5   | 0.86 ± 0.62 |
| p-value             | 0.45                | 0.92 | 0.14   | 0.15        | 0.9             | 0.74            | 0.58            |
| IVSI-110(G > A)/wt  | \( \alpha / \alpha \) | 180  | 93/87  | 27.2 ± 6.5 | 64.8 ± 5.3   | 20.6 ± 1.7   | 12.01 ± 1.28 | 59.1 ± 0.65 | 94.5 ± 1.9   | 4.5 ± 0.6   | 0.96 ± 0.81 |
| p-value             | 0.52                | 0.33 | 0.94   | 0.41        | 0.31           | 0.67            | 0.019           |
| IVSI-110(G > A)/wt  | \( \alpha / \alpha \) | 3    | 2/1    | 25.6 ± 3.2 | 66.9 ± 10.9 | 21.7 ± 4.9   | 12.1 ± 2.4   | 5.6 ± 0.25  | 93.3 ± 2.7   | 4.3 ± 1.02  | 2.3 ± 1.7   |
| p-value             | 0.52                | 0.33 | 0.94   | 0.41        | 0.31           | 0.67            | 0.019           |
| IVSI-111(G > A)/wt  | \( \alpha / \alpha \) | 111  | 60/51  | 28.4 ± 5.2 | 63.02 ± 3.73 | 19.21 ± 1.37 | 11.47 ± 1.20 | 56.3 ± 0.73 | 94.38 ± 2.90 | 4.46 ± 0.9  | 1.28 ± 0.99 |
| p-value             | 0.45                | 0.92 | 0.14   | 0.15        | 0.9             | 0.74            | 0.58            |
| IVSI-111(G > A)/wt  | \( \alpha / \alpha \) | 3    | 1/2    | 24 ± 2.0   | 77.2 ± 0.32  | 26.3 ± 1.2   | 15.6 ± 0.97  | 5.93 ± 0.2   | 95.7 ± 0.5   | 3.5 ± 0.25  | 0.5 ± 0.2   |
| p-value             | 0.45                | 0.92 | 0.14   | 0.15        | 0.9             | 0.74            | 0.58            |
| IVSI-5(G > C)/wt    | \( \alpha / \alpha \) | 43   | 21/19  | 26.1 ± 4.7 | 63.29 ± 3.80 | 19.42 ± 1.09 | 11.59 ± 1.30 | 6.03 ± 0.7   | 94.58 ± 0.88 | 5.03 ± 0.71 | 0.76 ± 0.60 |
| p-value             | 0.45                | 0.92 | 0.14   | 0.15        | 0.9             | 0.74            | 0.58            |
| IVSI-5(G > C)/wt    | \( \alpha / \alpha \) | 144  | 78/66  | 27 ± 5.4  | 65.2 ± 4.7   | 20.1 ± 1.9   | 11.9 ± 1.4   | 5.8 ± 0.6   | 94.9 ± 0.7   | 4.2 ± 0.6   | 0.76 ± 0.5  |
| p-value             | 0.45                | 0.92 | 0.14   | 0.15        | 0.9             | 0.74            | 0.58            |
| IVSI-5(G > C)/wt    | \( \alpha / \alpha \) | 1    | 0/1    | 26       | 67            | 21.5 ± 4.9   | 9.0           | 5.71        | ?             | ?             | ?             |
| p-value             | 0.45                | 0.92 | 0.14   | 0.15        | 0.9             | 0.74            | 0.58            |
| Initiation CD(T > C)/wt | \( \alpha / \alpha \) | 40   | 16/24  | 26 ± 3.1  | 73.74 ± 8.8  | 23.07 ± 3.3  | 12.72 ± 1.29 | 5.56 ± 0.7  | 96.35 ± 1.46 | 3.15 ± 1.3  | 0.5 ± 0.4   |
| p-value             | 0.45                | 0.92 | 0.14   | 0.15        | 0.9             | 0.74            | 0.58            |
| Initiation CD(T > C)/wt | \( \alpha / \alpha \) | 1    | 0/1    | 26       | 54.6          | 17.4 ± 4.9   | 12.6          | 7.25        | ?             | 5.8          | ?             |
| p-value             | 0.45                | 0.92 | 0.14   | 0.15        | 0.9             | 0.74            | 0.58            |
| CD39 (C > T)/wt     | \( \alpha / \alpha \) | 63   | 32/31  | 28.4 ± 6.8 | 62.7 ± 6.74  | 19.65 ± 1.3  | 11.42 ± 1.28 | 5.85 ± 0.69 | 94.4 ± 1.26 | 4.63 ± 1.2  | 0.86 ± 0.72 |
| p-value             | 0.45                | 0.92 | 0.14   | 0.15        | 0.9             | 0.74            | 0.58            |
| CD39 (C > T)/wt     | \( \alpha / \alpha \) | 1    | 0/1    | 22       | 61.6          | 20.1 ± 4.9   | 11.5          | 5.73        | 93.3         | 6.1          | 0.6         |
| p-value             | 0.45                | 0.92 | 0.14   | 0.15        | 0.9             | 0.74            | 0.58            |
| Hb 5/wt             | \( \alpha / \alpha \) | 400  | 220/180| 25.85 ± 4.5 | 83.73 ± 4.32 | 28.22 ± 1.97 | 13.78 ± 1.71 | 4.86 ± 0.56 | 57.11 ± 4.83 | 2.65 ± 0.81 | 0.61 ± 0.53 |
| p-value             | 0.45                | 0.92 | 0.14   | 0.15        | 0.9             | 0.74            | 0.58            |
| Hb 5/wt             | \( \alpha / \alpha \) | 4    | 1/3    | 24.3 ± 10.0| 85.95 ± 6.5  | 27.5 ± 1.34  | 13.96 ± 0.86 | 5.17 ± 0.11 | 56.72 ± 2.27 | 3.12 ± 0.38 | 0.27 ± 0.38 |
| p-value             | 0.45                | 0.92 | 0.14   | 0.15        | 0.9             | 0.74            | 0.58            |
The frequency of the alpha-globin gene triplication is varied, and it is dependent on the prevalence of thalassemia disease and some selection mechanisms such as endemic malaria in the studied countries [3,10].

The highest carrier frequency of the anti 3.7 mutation was observed in 4.0% in healthy individuals of Indian, and lowest carrier frequency was reported in Malay population with 0.59%. In this study from the 2000 healthy individuals, 18 had alpha triplication mutation, it can be stated that the frequency of this allele in healthy people in Khuzestan province, particularly in Ahwaz city, equals 0.9% which is lower than previous Iranian study and more than the other countries (Table 2). The following table shows the prevalence of this triplication in the healthy individuals in Iran and other populations of the world.

Our results also showed that the frequency of the alpha-globin gene triplication in the Khuzestan population including hemoglobinopathies and normal individuals is 1.39%; this result is close to the results obtained from our country in previous studies. The lowest carrier frequency of alpha-globin gene triplication including hemoglobinopathies and normal individuals was observed in Omani with 0.47%, whereas the higher frequencies were in other populations, including 10% in Mexican, Saudi Arabian 3.9% and 3.1% in North Indian population (Table 3).

In this study, we also investigated the average hematological parameters of every studied genotype with alpha gene triplication in comparison with the same mutations with the normal of the alpha-globin gene as well. We did not find any significant role in the changing of blood indices, only a marginal difference in beta-thalassemia carriers if any (Table 1). We have just had four beta-thalassemia carriers with alpha triplication lower than 9 g/dl of hemoglobin (one female CD36/37-T/wt, 7.9 g/dl, one male IVSII-I (G-A)/ wt, 8.6 g/dl, one female CD82-83(-G)/ wt, 8.5 g/dl, one female CD8(-AA)/ wt, 8.6 g/dl); none of them received blood transfusion.

The general idea about the additional α gene is that the extra α gene aggravates the mild phenotype of the β-thal carrier to the thalassemia intermedia of mild severity, but in most studies, the genotype and phenotype correlation between coinheritance of heterozygous β-thal and the α-globin triplication remains unclear [11–14].

Previous studies showed that HbA2 and fetal hemoglobin levels were increased and very significantly reduced Hb level in α-thal carriers with six different mutations and in association with β-thalassemia [12,15,16]. We have not observed a significant role in phenotype and hematological indices in individuals who carried different heterozygous β-globin gene mutations with the α-globin triplication.

Although we expected that extra chains of α globin gene eliminated by proteolysis did not have a significant effect or very limited effect in phenotype and hematological parameters.

In this study, our result was similar to some previous reports [10,15,17,18]. For instance, Giordano et al found that none of the 12 β-thal carriers with six different mutations and in association with an α-triplication had a history of blood transfusion. Xiong et al also described 74 individuals co-inheritance β thalassemia in carrier status and α-triplication all presented the phenotype of the β-thal trait. Our observations are precisely in keeping with the result that the presence of a triplicated α-globin allele in β-thalassemia heterozygotes is associated with a phenotype of β-thalassemia.

### TABLE 2: Allele and genotype frequency of αααanti3.7 in normal subjects in this study and other populations

| Population     | Number of chromosomes studied | Allele frequency of αααanti3.7 (%) | Genotype frequency of αααanti3.7 (%) | References |
|----------------|--------------------------------|-----------------------------------|-------------------------------------|------------|
| Normal subjects|                                |                                   |                                     |            |
| Mexican        | 84                             | 0.01                              | 2                                   | [20]       |
| Togolese       | 342                            | 0.01                              | 2.4                                 | [20]       |
| Kenyan         | 114                            | 0.008                             | 1.6                                 | [20]       |
| South African blacks | 306                    | 0.01                              | 1.96                                | [20]       |
| Cypriote       | 990                            | 0.01                              | 2                                   | [20]       |
| Namibian       | 202                            | 0.01                              | 1.98                                | [20]       |
| Portuguese     | 200                            | 0.020                             | 4                                   | [20]       |
| Indian         | 1856                           | 0.004                             | 0.75                                | [21]       |
| Indian         | 536                            | 0.011                             | 2.2                                 | [22]       |
| Indian         | 2550                           | 0.02                              | 4                                   | [23]       |
| North Indian   | 416                            | 0.017                             | 3.36                                | [24]       |
| Chinese        | 500                            | 0.008                             | 1.6                                 | [20]       |
| Southern Chinese | 2338                  | 0.0047                            | 0.94                                | [25]       |
| Chinese        | 1020                           | 0.0098                            | 1.96                                | [22]       |
| Malay          | 1014                           | 0.003                             | 0.59                                | [22]       |
| Thai           | 430                            | 0.007                             | 1.39                                | [26]       |
| North Morocco  | 3316                           | 0.0006                            | 0.12                                | [27]       |
| Iranian        | 794                            | 0.01                              | 2                                   | [28]       |
| Our study      | 4000                           | 0.0045                            | 0.9                                 |            |

### TABLE 3: Total frequency of αααanti3.7 in this study and other populations including hemoglobinopathies and normal individuals

| Population     | Individuals studied | Genotype frequency of αααanti3.7 (%) | References |
|----------------|---------------------|-------------------------------------|------------|
| Turkish        | 225                 | 5 (2.2%)                            | [33]       |
| Saudi Arabian  | 104                 | 4 (3.9%)                            | [34]       |
| Omani          | 634                 | 3 (0.47%)                           | [35]       |
| North Indian   | 419                 | 13 (3.1)                            | [24]       |
| Indian         | 1253                | 15 (1.1)                            | [21]       |
| Dutch          | 3500                | 42 (1.2%)                           | [10]       |
| Mexican        | 109                 | 11 (10%)                            | [20]       |
| Iranian        | 4010                | 69 (1.7%)                           | [14]       |
| Iranian        | 1700                | 20 (1.2%)                           | [19]       |
| Our study      | 6404                | 84 (1.31%)                          |            |
Previous studies reported similar genotypes of our studies from patients with triplicated \( \alpha \)-globin genes and heterozygous \( \beta \)-thalassemia

| Population | \( \beta \)-genotype | Number of patients | Hb (g/dl) Mean ± SD | Hb Less than 9 (g/dl) | Transfusion-dependent |
|------------|----------------------|--------------------|---------------------|-----------------------|-----------------------|
|            |                      |                    |                     |                       | No. of patients       |
|            |                      |                    |                     |                       | Regular | Irregular | Splenectomy | References |
| Iranian    | IVSII-1(G-A)/wt      | 18                 | 9.65 ± 0.2          | ?                     | 14 | 6 | 8 | 2 | [14] |
| Iranian    | IVSII-1(G-A)/wt      | 5                  | 8.56 ± 0.7          | 3                     | 4 | 2 | 2 | 2 | [19] |
| Italian    | IVSII-1(G-A)/wt      | 3                  | 11 ± 1.12           | 0                     | 0 | 0 | 0 | 0 | [29] |
| Brazilian  | IVSII-1(G-A)/wt      | 2                  | 10.2 ± 1.6          | 0                     | 0 | 0 | 0 | 0 | [11] |
| Our study  | IVSII-1(G-A)/wt      | 9                  | 10.65 ± 1.4         | 1                     | 0 | 0 | 0 | 0 |          |
| Total      | IVSII-1(G-A)/wt      | 37                 | 9.94 ± 1.43         | 4                     | 18 | 8 | 10 | 4 |          |
| Total excluding [14,19] | IVSII-1(G-A)/wt | 14                 | 10.76 ± 1.26        | 1                     | 0 | 0 | 0 | 0 |          |
| Iranian    | CD15(TGG-TGA)/wt     | 1                  | 8.2                 | 1                     | 1 | 1 | 0 | 1 | [14] |
| Our study  | CD15(TGG-TGA)/wt     | 2                  | 10.9 ± 1.55         | 0                     | 0 | 0 | 0 | 0 |          |
| Total      | CD15(TGG-TGA)/wt     | 3                  | 9.55 ± 1.9          | 1                     | 1 | 1 | 0 | 1 |          |
| Greek CD39 (C > T)/wt | 4                  | 10.4 ± 1.1         | 0                     | 0 | 0 | 0 | 0 | [15] |
| European ancestry | CD39 (C > T)/wt | 5                  | 9.1 ± 0.84          | 2                     | 2 | 1 | 1 | 0 | [30] |
| ? CD39 (C > T)/wt | 9                  | 9.2 ± 1.6          | 4                     | 4 | 0 | 4 | 2 | [3] |
| Italian    | CD39 (C > T)/wt      | 18                 | 9.93 ± 1.01         | 4                     | 3 | 0 | 3 | 3 | [29] |
| Italian    | CD39 (C > T)/wt      | 1                  | 8.6                 | 1                     | 0 | 0 | 0 | 0 | [31] |
| Dutch CD39 (C > T)/wt | 15                 | 10.1               | ?                     | 0 | 0 | 0 | 0 | [10] |
| Greece CD39 (C > T)/wt | 6                  | 9.86 ± 1.67        | 2                     | 2 | 0 | 2 | ? | [36] |
| Our study  | CD39 (C > T)/wt      | 1                  | 11.5                | 0                     | 0 | 0 | 0 | 0 |          |
| Total CD39 (C > T)/wt | 59                 | 9.31 ± 1.22        | 13                    | 11 | 1 | 10 | 5 |          |
| ? IVSI-110(G > A)/wt | 3                  | 10.1 ± 2.3         | 1                     | 0 | 0 | 0 | 0 | [3] |
| Italian    | IVSI-110(G > A)/wt   | 1                  | 8.9                 | 1                     | 0 | 0 | 0 | 1 | [29] |
| Gypsy      | IVSI-110(G > A)/wt   | 1                  | 8.5                 | 1                     | 0 | 0 | 0 | 0 | [32] |
| Dutch      | IVSI-110(G > A)/wt   | 10                 | 11.2                | ?                     | 0 | 0 | 0 | 0 | [10] |
| Greece     | IVSI-110(G > A)/wt   | 2                  | 8.7 ± 0.28          | 2                     | 0 | 0 | 0 | 0 | [36] |
| Our study  | IVSI-110(G > A)/wt   | 1                  | 10.2                | 0                     | 0 | 0 | 0 | 0 |          |
| Total      | IVSI-110(G > A)/wt   | 18                 | 9.6 ± 1.0           | 5                     | 0 | 0 | 0 | 0 |          |
| Iranian    | IVSI-5(G > C)/wt     | 3                  | 7.9                 | 3                     | 2 | 1 | 1 | 1 | [14] |
| Iranian    | IVSI-5(G > C)/wt     | 2                  | 8 ± 0.42            | 2                     | 2 | 1 | 1 | 1 | [19] |
| Iranian    | IVSI-5(G > C)/wt     | 2                  | 12.1 ± 1.7          | 0                     | 0 | 0 | 0 | 0 | [28] |
| ? IVSI-5(G > C)/wt | 2                  | 10.8 ± 0.5         | 0                     | 0 | 0 | 0 | 0 | [3] |
| Our study  | IVSI-5(G > C)/wt     | 1                  | 9.0                 | 0                     | 0 | 0 | 0 | 0 |          |
| Total      | IVSI-5(G > C)/wt     | 10                 | 9.56 ± 1.83         | 5                     | 4 | 2 | 2 | 2 |          |
| Total excluding [14,19] | IVSI-5(G > C)/wt | 5                  | 10.63 ± 1.55        | 0                     | 0 | 0 | 0 | 0 |          |
| ? IVSI-1(G > A)/wt | 2                  | 9 ± 1.4            | 1                     | 0 | 0 | 0 | 1 | [3] |
| Greek      | IVSI-1(G > A)/wt     | 8                  | 9.2 ± 1.3           | 3                     | 0 | 0 | 0 | 0 | [15] |
| Greek      | IVSI-1(G > A)/wt     | 7                  | 9.1 ± 1.8           | 4                     | 4 | 0 | 4 | ? | [36] |
| Iranian    | IVSI-1(G > A)/wt     | 2                  | 10.1                | ?                     | 2 | 1 | 1 | 1 | [14] |
| Italian    | IVSI-1(G > A)/wt     | 6                  | 10.58 ± 1.2         | ?                     | 0 | 0 | 0 | 1 | [29] |
| Our study  | IVSI-1(G > A)/wt     | 1                  | 10.6                | 0                     | 0 | 0 | 0 | 0 |          |

(Continues)
TABLE 4 (Continued)

| Population | β-genotype | Number of patients | Hb (g/dl) | Hb Less than 9 (g/dl) | Transfusion-dependent |
|------------|------------|--------------------|-----------|-----------------------|----------------------|
|            |            |                    | Mean ± SD | No. of patients | Regular | Irregular | Splenectomy | References |
| Total      | IVSI-I(G>A)/wt | 26                  | 9.76 ± 1.0 | 8         | 6 | 1 | 5 | 3 |
| Total excluding [14] | IVSI-I(G>A)/wt | 24                  | 9.70 ± 0.9 | 8         | 4 | 0 | 4 | 2 |
| Iranian    | CD44(C)/wt  | 1                   | 9.6       | 0         | 0 | 0 | 0 | 0 | [14]
| Iranian    | CD44(C)/wt  | 1                   | 9.8       | 0         | 0 | 0 | 0 | 0 | [14]
| Our study  | CD44(C)/wt  | 5                   | 10.4 ± 0.1| 0         | 0 | 0 | 0 | 0 |
| Iranian    | CD8(-AA)/wt | 2                   | 9.4 ± 3.7 | 1         | 1 | 1 | 0 | 1 | [14]
| Iranian    | CD8(-AA)/wt | 3                   | 10.2 ± 1.1| 0         | 0 | 0 | 0 | 0 | [14]
| Iranian    | CD36/37-T/wt| 3                   | 8.5 ± 1.83| 2         | 2 | 1 | 1 | 0 | [14]
| Iranian    | CD36/37-T/wt| 1                   | 10.1      | 0         | 0 | 0 | 0 | 0 | [14]
| Our study  | CD36/37-T/wt| 15                  | 10.5 ± 1.6| 1         | 0 | 0 | 0 | 0 |
| Iranian    | Indian deletion/wt | 1           | 7.3       | 1         | 1 | 1 | 0 | 0 | [14]
| Our study  | Indian deletion/wt | 3           | 12.2 ± 0.2| 0         | 0 | 0 | 0 | 0 |
| Dutch      | CD5(-CT)/wt | 11                  | 8.7       | ?         | 0 | 0 | 0 | 0 | [10]
| Our study  | CD5(-CT)/wt | 1                   | 10.2      | 0         | 0 | 0 | 0 | 0 |
| Dutch      | IVSI-6(T-C)/wt | 15            | 14        | 0         | 0 | 0 | 0 | 0 | [10]
| Our study  | IVSI-6(T-C)/wt | 2            | 12.2 ± 0.3| 0         | 0 | 0 | 0 | 0 |
| Total      |              | 218                 | 9.9 ± 1.2 | 42        | 45 | 17 | 28 | 17 |
| Total excluding [14,19] |              | 177                | 10.3 ± 1.0| 28        | 15 | 1 | 14 | 7 |

minor. This information is of importance in terms of genetic counseling.

In this study, we also evaluated previous data reported similar genotypes of our studies from patients with triplicated α-globin genes and heterozygous β-thalassemia for Hb (g/dl), transfusion-dependent, and splenectomy. Of 37 affected persons who coinheritance of the β-thalassemia heterozygotes (IVSII-I (G-A)/wt) with triplicated alpha-globin genes, 18 (48.6%) were transfusion-dependent, four underwent splenectomy, and four had Hb less than 9 g/dl. In comparison with previously reported mutations, the results of previous Iranian studies [14,19] reported from the same centers were different significantly. If we exclude these studies [14,19], the results are changed fundamentally, where no one of individuals coinheritance of the β-thalassemia heterozygotes (IVSI-I (G-A)/wt) with triplicated alpha-globin genes, 18 (48.6%) were transfusion-dependent, four underwent splenectomy, and four had Hb less than 9 g/dl. It is suggested that the results of these two articles [14,19] necessary to be re-evaluated by a hematologist-oncologist and verification of hematology analyzers (automated blood cell counters) are required.

In addition, of 59 β-thalassemia heterozygote (CD39 (C>T)/wt) with triplicated alpha-globin genes, 11 (18.64%) were transfusion-dependent, five (8.5%) underwent splenectomy, and 13 (22.0%) had Hb less than 9 g/dl. Due to comparing reported mutations in the previous studies, it can be recommended that the mutation of codon 39 needs to be taken into consideration and can have a clinical effect (Table 4).

Finally, we evaluated all reported mutations in this and previous studies, for Hb (g/dl), transfusion-dependent, and splenectomy. Of 218 β-thalassemia heterozygote with triplicated alpha-globin genes, 45 (20.64%) were transfusion-dependent, 17 (7.8%) underwent splenectomy, and 42 (19.26%) had Hb less than 9 g/dl.

If we omit these studies [14,19], the results are altered. The total numbers of studies persons are reduced to 177. Of 177 affected persons, 15 (8.47%) were transfusion-dependent, seven (3.95%) underwent splenectomy, and 28 (15.82%) had Hb less than 9 g/dl where most of them coinheritance of the β-thalassemia heterozygotes (CD39 (C>T)/wt) with triplicated alpha-globin genes. The data are summarized in Table 4.

In conclusion, the genotype of triplicated α-globin gene and heterozygosity for β-thalassemia mutation is not necessary to be considered as a cause of β-thalassemia intermedia in our locality. Therefore, it is not essential to offer a prenatal diagnosis test to families and couples carrying an α-globin gene triplication and a heterozygosity for β-thalassemia. Therefore, not every individual with triplicated alpha globin coherited with beta-thalassemia trait will have a significantly lower Hb than normal, and it is highly likely that none of them will need transfusion. Due to previous studies, it can be recommended that the mutation of codon 39 needs to be taken into consideration.
ACKNOWLEDGMENTS
We would like to thank all patients for their contributions to the study.

CONFLICT OF INTEREST
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

AUTHOR CONTRIBUTIONS
Mohammad Hamid directed the project, collected data, performed analysis, and wrote the manuscript. Bijan keikhaei, Alihossein Saberi, Gholamreza Shariati, Hamid Galehdari, Marziye Mohammadi-Anaei provided the samples and clinical data. All of the authors reviewed and gave the final approval for the paper.

DATA AVAILABILITY STATEMENT
All data generated during and/or analyzed during the current study are available upon request by contact the corresponding author.

FUNDING INFORMATION
This study was supported by grant number 687 from the Pasteur Institute of Iran, Tehran, Iran.

ORCID
Mohammad Hamid https://orcid.org/0000-0002-4625-0713

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How to cite this article: Hamid M, keikhaei B, Galehdari H, Saberi A, Sedaghat A, Shariati G, et al. Alpha-globin gene triplication and its effect in beta-thalassemia carrier, sickle cell trait, and healthy individual. eJHaem. 2021;2:366–374. https://doi.org/10.1002/jha2.262