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Original Research

Alterations on high HbF levels may be associated with KLF1 gene mutations

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Abstract: The KLF1 gene synthesizes a transcription factor in the zinc finger structure that regulates the transcription of β-, γ-globin, and Foxm1 genes. This factor plays an important role in the erythropoiesis mechanism by modifying the chromatin structure and is involved in the regulation of transcription in the opening of the β-globin gene. β-globin gene expression could be disrupted by a mutation, which may be a possible cause of a disruption in regulation of the promotor of the β-globin gene where the KLF1 transcription factor binds. This can lead to an inherited high fetal hemoglobin (HbF) ratio in people. Therefore, the main aim of this study was to determine the effects of KLF1 mutations on these high levels of HbF. In this study, in order to determine the relationship between the KLF1 mutations and the high HbF levels three exons along with the 5'-UTR and 3'-UTR regions of the KLF1 gene were sequenced of 53 volunteers. In this study, 3 variations in the non-coding regions of the KLF1 gene were not associated with a high level of HbF. Five variations were detected in the second exon of KLF1 gene. One of these is a frame shift that occurs when GG bases are inserted between the 59-60 codons, and the other four variations occur as a base substitution variations. No correlation was found between high HbF levels and neutral variants. Only polar-nonpolar amino acid changes were found at two points. At one of them, a significant drop in the high HbF levels was observed, while the other was observed to be high near to the critical limit. These findings suggested that variations in function of the KLF1 gene can alter the HbF levels.

Key words: KLF1; Fetal hemoglobin; Globin genes; Variations.

Introduction

Hemoglobin (Hb) molecule, which is found in erythrocytes, has high oxygen binding ability and transports oxygen from the lungs to the tissues, and carbon dioxide and protons from the tissues to the lungs. In the quaternary structure, the hemoglobin molecule in erythrocytes weighs 64,500 daltons (1,2).

The HbA molecule is a tetrameric metalloprotein that constitutes a chain of two alpha (α) and two beta (β) globin chains. In healthy adults, the quantity of HbA (α2β2) is estimated to be 97%. Fetal hemoglobin (HbF) is the major hemoglobin in human fetus that transport the oxygen during the last seven months of development in the uterus and about six months after birth. HbF is composed of two alpha and two gamma chains (α2γ2), which is estimated to be below 1% in adults. However, HbA2 (α2δ2), which has two alpha and two delta (δ) chains in its structure, is ranged between 2-3% in adults. Alpha-like chain zeta (ζ), beta-like chains epsilon (ε), and gamma (γ) chains are found in the structure of Hb Gower [Hb Gower I (ζ2ε2) and Hb Gower II (α2ε2)] and Hb Portland (ζ2γ2). These are synthesized in early embryonic life (3-8).

Hemoglobinopathies are the phenomena characterized by a decrease in the synthesis of the globin chains found in the hemoglobin structure or by amino acid changes in their structures. Later on, the same syndrome, which is the result of the reduction or absence of the synthesis of globin chains, is referred as thalassemia. The pathophysiology of thalassemia is caused by the imbalance of the globin chain ratio. The other chain produced in normal ratios is relatively oversized; to form the tetramer in the absence of the complemennter chain, it precipitates in the premature red blood cells and cause damage to the cell membrane. Additionally, the amount of Hb in the red cells decreases and causes hypochromic microcytic anemia. The amino acid change in the globin chain causes to abnormal hemoglobin or Hb variants. The fetal hemoglobin, which is composed of two alpha and two gamma chains ($\alpha_2\gamma_2$), functions as the basic oxygen transport protein in the 2nd and 3rd trimester in fetus, but it is decreased immediately after birth to less than 1%. However, in some people, genetically HbF maintains its high level after birth.

The KLF1 (EKLF) gene (EKLF: Erythroid Kruppel-Like Factor1) encodes a transcription factor in the zinc finger structure (9,10). This transcription factor modifies the chromatin structure and plays an important role in the mechanism of erythropoiesis and takes a role in the regulation of transcription in the opening of the β-globin gene. The site where the KLF1 transcription factor binds is the promotor of the β-globin gene. A possible mutation causes disruption in the all regulation process and disrupt the gene expression of β-globin. This leads to an inherited high HbF levels in individuals (10,11). The increased HbF ratio in adult thalassemia patients alleviates the course of the disease, which is counted to the advantage of the patient but not as disad-

Table 1. Primer sequences used for amplification of KLF1 exons and their amplicons lengths (Radmilovic et al., 2013).

Primer	Primer sequence (5'-3')	Amplicon length (bp)
KLF1_ex1_FW	GCTTTGGACACAGGGTTAGT	400
KLF1_ex1_RV	TCAGGTCAAGATGCAGGTCT	
KLF1_ex2a_FW	TTCCAAAGCCTCTGCGTCAG	570
KLF1_ex2a_RV	ACGCCGCAGGCACTGAAAG	
KLF1_ex2b_FW	GGGAGGAAGAGGACGATGA	985
KLF1_ex2b_RV	GGACAAGGAAGCCATAAGC	
KLF1_ex3_RV	ACCTTCAGGAGCCGCTTTCT	650
KLF1_ex3_FW	AGGCTGAGTAAAGGGGTGTG	

vantage. Mutations in the β-globin gene promotor, particularly in regions where the KLF1 transcription factor is bound, are known to be responsible for the elevation of the HbF level (9,11-14). This is an advantage in thalassemia patients. The same conclusions apply to mutations detected in the γ-globin gene promotor (15). Additionally, it was determined that chromatin remodeling factors also had an effect on high HbF ratios, and possible mutations in these factors reduced the expression of delta-beta hemoglobin (16). KLF1 functions as a key for both gamma and beta-globin genes by regulating the BCL11A gene (12,17,18). Wang et al. (2013) reported that KLF1 gene mutations play an important role in relation to high levels of HbF in a Chinese population, and these KLF1 mutations were not found in individuals with normal HbF levels (12). The KLF1 or γ-globin gene promotor mutation has been found to play an important role in the level of HbF (13,15). De Andrade et al. (2006) reported that the chromatin remodeling factors ARIDIP and TSPYL1 play a crucial role in high HbF levels down-regulating the delta-beta hemoglobin expression (16). Therefore, the main aim of this study was set up to determine the cause of the increase in the rate of HbF that alleviates the course of the disease, especially in patients with abnormal hemoglobin and β-thalassemia. In order to achieve this purpose, DNA sequences were determined by amplifying the exon regions of the KLF1 gene, variations within the gene were determined, and the association with high HbF was investigated in all of the individuals with high HbF levels.

Materials and Methods

The ethical approval was obtained from Ethics Committee of Non-Interventional Research (approval date and number: 06.05.2014/07) of Firat University, Elazığ, Turkey. The study was conducted in agreement with the statement on the Declaration of Helsinki.

A total of fifty-three samples with HbF level greater than 1.5% were examined in this study. Genetic material of the fifty-three samples were isolated using whole blood by ROCHE DNA isolation kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer instructions. The primers that were used for amplification of the targeted genes are listed in Table 1.

After the PCR, the amplified PCR products were electrophoresed using a 2% agarose gel, stained with ethidium bromide, and then photographed on a gel imaging system. DNA sequence analysis of well-screened samples was performed using an automated chain sequencing based on Sanger's enzymatic DNA synthesis. Bi-directional sequencing was performed in order to ensure any possible error. After the DNA Sequencing analysis, genotypes of the samples were determined using the BioEdit Sequence Alignment program (version 7.0.8.0). Sequence results were checked from the NCBI database by comparing both the 5' and 3' ends (https://www.ncbi.nlm.nih.gov/nucleotide; BCLA11A NCBI Reference Sequence: NG_011968.1; KLF1 NCBI Reference Sequence: NG_013087.1).

Statistical analysis was performed using the SPSS package program (version 16.0, SPSS Inc., Chicago, IL).

Results

The KLF1 gene is located on the 19th chromosome (19p13.13). It is composed of three exons and two introns, and synthesizes a polypeptide chain with a total of 362 amino acids.

In this study, a total of 2140 bp were sequenced and analyzed including exons and 5'-UTR and 3'-UTR sequences. The first exon encodes 29 amino acids that are 87 bases in length. The second exon encodes 825 bases, which codes 275 amino acids. The third exon with a base length of 174 encodes a total of 58 amino acids. According to the obtained results, three variations in the non-coding regions and 5 variations in the coding region of the KLF1 gene were detected. One of the variations in non-coding regions is the G/A substitution type variation at -85 localization in the 5'-UTR region (Figure 1). The heterozygous G/A variation in this region was found in only two individuals and no A/A homozygous variation was found. Fifty-one people have normal homozygous G/G base sequence. Another variation was detected in non-coding regions at 1st intron. Here, a G base was added in this part. While CCGAA was the regular sequence, a CCGGAA sequence was detected in one person (Figure 2).

One variation was found in the 3'-UTR region. This variation occurs as a result of the exchange of G base with A base (G/A). Of the fifty-three patients, a total of eleven individuals was found with heterozygous G/A

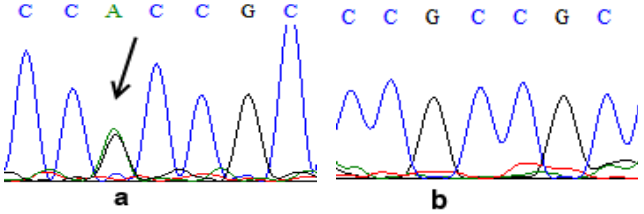

Figure 1. Variations at -85 localization of the 5'-UTR region in the KLF1 gene a) G/A heterozygous, b) G/G homozygous.

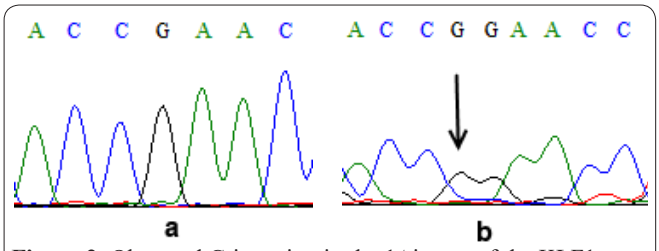

Figure 2. Observed G insertion in the 1st intron of the KLF1 gene a) Normal sequence, b) G insertion.

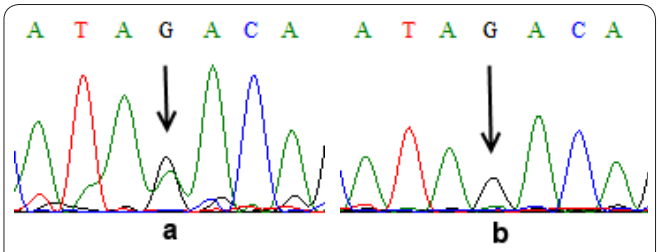

Figure 3. The observed G/A variation in 3'-UTR region of KLF1 gene; a) heterozygous G/A, b) normal sequence.

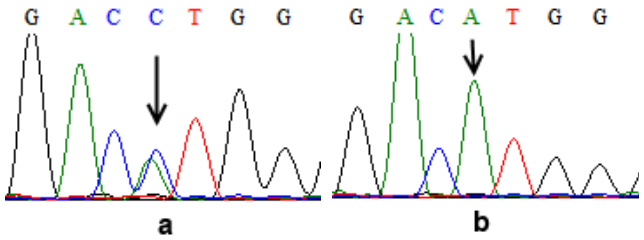

Figure 4. The change of ATG (met) to CTG (leu) by A/C variation; a) heterozygous A/C, b) normal sequence.

and none with A/A homozygotes (Figure 3).

All of the 5 variations, which were identified in the coding region of the KLF1 gene, were detected in the 2 nd region, and no variation was found at 1st and 2nd exons. ATG (met), which is the 39th codon of the 2nd exon, was changed to CTG (leu) due to A/C type variation (Figure 4). While 5 individuals were determined to be A/C heterozygous, none was C/C homozygous.

Another change was observed in the 2nd exon, which is the frame shift type variation that occurs with the addition of two GG base in before the 60th codon (Figure 5). The 59th and the 60th codons encode proline and glutamic acid, respectively. However, after the addition of the GG-bases, the code was changed completely from the glutamic acid codon. In the later parts of the sequence, the 237th codon turned into a stop codon (TGA). This type of heterozygous abnormality was determined in only one person.

Another variation detected in the 2nd exon of the KLF1 gene was the T/C substitution which resulted in a change in 102nd codon (TCC) to CCC codon. Normally, this codon encodes the serine amino acid, but transform into a proline coding region. Twenty-three individuals were determined to be T/C heterozygous and three were C/C homozygous (Figure 6).

The T/C variation was also found in the 182nd codon in the 2nd exon of the KLF1 gene. In the normal case, 182nd codon (TTC) encodes phenylalanine after the T/C change it transforms to CTC codon and encodes the leucine amino acid. This T/C heterozygous variation was detected in two individuals while none C/C homozygous were found (Figure 7).

Another variation detected in the second exon of the

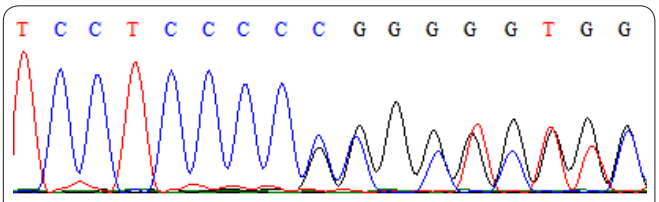

Figure 5. The GG insertion after the 59th codon of the 2nd exon in KLF1 gene.

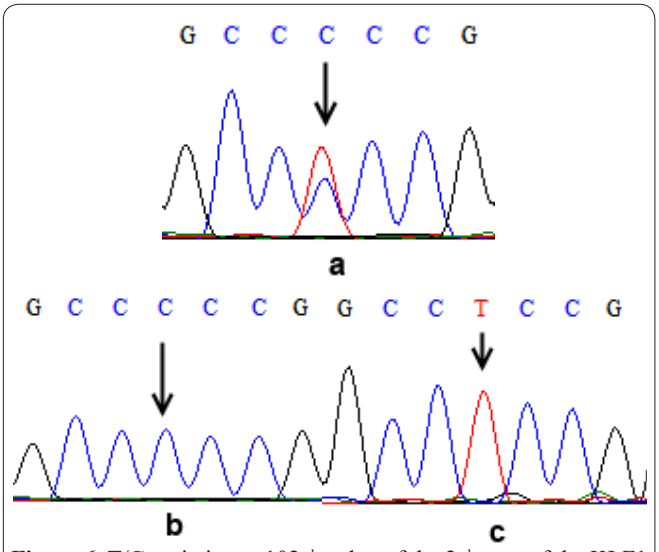

Figure 6. T/C variation at 102nd codon of the 2nd exon of the KLF1 gene; a) heterozygous T/C, b) homozygous C/C, c) normal sequence (homozygous T/T).

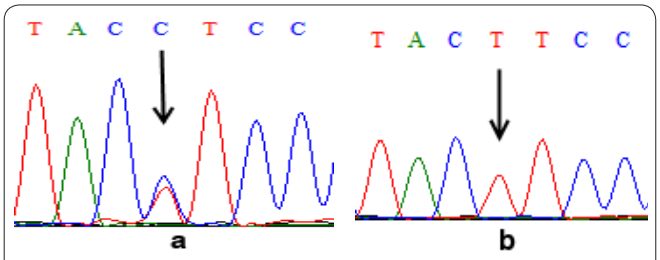

Figure 7. T/C variation at the 182nd codon of the 2nd exon in the KLF1 gene; a) heterozygous T/C, b) homozygous T/T.

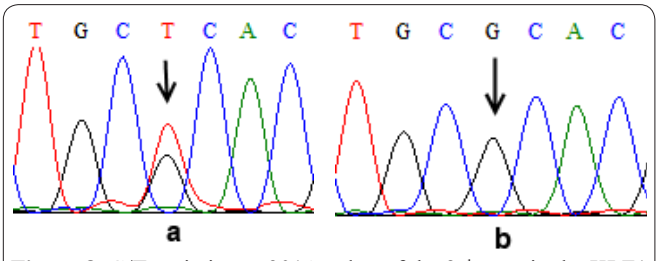

Figure 8. G/T variation at 301st codon of the 2nd exon in the KLF1 gene; a) heterozygous G/T, b) homozygous G/G.

KLF1 gene was a G/T variation, which allows the amino acid change of the arginine encoded by the 301st codon to be leucine aminoacid. The 301st codon was CGC and it became CTC. This variation was determined in only two individuals (Figure 8). According to the statistical analysis results, it was determined that none of the variations detected in the non-coding regions of KLF1 were associated with high HbF values (Table 2).

Analysis results indicated that the codon 39 A/C, codon 59 GG insertion, codon 102 T/C, codon 182 T/C, and codon 301 G/T type variations were not associated with high HbF levels (Table 2). However, when T/C heterozygotes and C/C homozygotes were compared

Table 2. The statistical significance between HbF value and variations of KLF1gene.

Locations	Genetic Variations	N	HbF mean	Standard deviation	Standard error	F	Sig. P
5-'UTR, -85 G/A	GG	51	2.0039	.53701	.07520	2.794	.101
	GA	2	2.0000	.00000	.00000		
1st intron, G insertion	Normal	52	2.0115	.52865	.07331	--	--
	G ins.	1	1.6000	--	--		
3'-UTR G/A substitution	GG	41*	2.0366	.56822	.08874	1.303	.259
	GA	11	1.9091	.35624	.10741		
Codon 39, A/C	AA	48	1.9958	.53553	.07730	.023	.880
	AC	5	2.0800	.47645	.21307		
Codon 59, GG insertion	Normal	52	2.0038	.53172	.07374	--	--
	GG ins.	1	2.0000	--	--		
Codon 102, T/C	TT	27	2.1000	.63971	.12311	1.513	.230
	TC	23	1.9435	.36535	.07618		
	CC	3	1.6000	.10000	.05774		
Codon 182, T/C	TT	51	2.0157	.53193	.07449	.670	.417
	TC	2	1.7000	.28284	.20000		
Codon 301, G/T	GG	51	1.9765	.49258	.06898	3.556	.065
	GT	2	2.7000	1.13137	.80000		

Table 3. The statistical significance between HbF value and variations in codon 102 of KLF1gene according to over-dominant genetic model

Codon102 T/C substitution		N	Mean	Standard deviation a	Standard error	Levene's Test for Equality of Variances	
						F	Significance P
HbF	TT	TT	2.1000	.63971	.12311	5.910	.019
	TC+CC	TC+CC	1.9435	.36535	.07618		

against TT homozygotes, it was observed that the HbF values decreased significantly (Table 3). Additionally, all variation types of the volunteers, comparison of HbA2 and HbF values, and hematologic data are presented in Table 4.

Discussion

In this study, 3 variations in the non-coding regions of the KLF1 gene were not associated with high HbF levels. The same situation could also be said for the variations detected in the coding regions. The T/C substitution, which was investigated in the 102nd codon area, is the only substitution that caused a decrease in the HbF level. However, the G/T substitution in the 301st codon region can be associated with high HbF level (P=0.06).

Four of five variations in the coding region were formed as point mutations. When the amino acid changes were examined, nonpolar and neutral amino acids (met and phe, respectively) were replaced with other nonpolar and neutral amino acids (leu and leu, respectively) in 39th and 182nd codons. This probably did not have any effect on protein structure and function. In the 102nd and 301st codons, polar neutral and polar positive amino acids (ser and arg, respectively) were changed to nonpolar and neutral amino acids (pro and leu, respectively). The more polar-nonpolar exchange is more important than the charge exchange in amino acids of the same polypeptide chain. There are two important structures that stabilizes the tertiary structure of a polypeptide chain. The first one is S-S the bridges, and the second is polar-nonpolar amino acids. Therefore, these two variations can be linked to the HbF level. The low number of individuals carrying these variations, which are only two heterozygous individuals carrying the variant causing the change at 301st codon, makes it particularly difficult to establish a clear relationship between variations and HbF levels. Likewise, the same case applies for the GG insertion in the 2nd exon. It was determined that most of the amino acids beginning from the 60th codon to the 237th codon was changed; and with change of the 237th codon to stop codon, this type of variation that synthesizes premature polypeptide was detected as heterozygote in only one person.

Shariati et al. (2016) studied the genetic disruption of the KLF1 gene to overexpress the γ-globin gene using the CRISPR/Cas9 system (19). They reported that 288th codon in the second exon and a point on the third exon of the KLF1 gene were important. In this study, we did not counter any variation in the 288th codon and third exon.

Analysis of an individual with a null mutation for the KLF1 gene reported that anemia and many phenotypic abnormalities were detected in this newborn individual and that the KLF1 gene regulated the KIF23 and KIF11 genes. These two genes produce the necessary polypeptides for proper and on time cytokinesis (20).

Vinjamur et al. (2014) have shown that KLF1 and KLF2 genes are key genes for the development of embryonic erythrocytes, and they have determined the pathways of action of these genes (21). According to the researchers, KLF1 and KLF2 genes have been reported to take a role in survival of normal peripheral blood cells and to conserve the amount of globin mRNA that is specific to erythrocyte cells. The KLF1 and KLF2 genes regulate many genes associated with proliferation such as Foxm1 in a coordinated fashion. The different expression of the Foxm1 gene causes the formation of anemia. In blood cells, the role of KLF1 is to bind to the promotor of the Foxm1 gene and regulate it. Mutations in the Gγ and Aγ-globin genes, other than the KLF1 and BCL11A genes, can induce (22-25) or reduce the level of HbF (26). In some cases, in individuals with high HbF levels who have variations in more than one loca-

Table 4. Variation types of the volunteers. Comparison of HbA2 and HbF values and hematologic data.

Donor no	KLF1								RBC	HGB	HCT	MCV	MCH	MCHC	A2	F
	5'-UTR G/A	1st intron G ins	3'-UTR G/A	39. codon A/C	60. codon GG ins	102. codon T/C	182. codon T/C	301. codon G/T								
0040	GG	N	GG	AA	N	TT	TT	GG	5.58	16.2	50.5	90.3	28.9	32.0	3.0	1.5
0066	GG	N	GG	AA	N	TT	TT	GG	4.94	14.2	45.0	91.2	28.7	31.5	3.0	1.8
0092	GG	N	GG	AA	N	TC	TT	GG	4.97	14.8	48.5	97.5	29.8	30.5	3.0	2.1
0123	GG	N	GG	AA	N	TC	TT	GG	5.01	14.2	39.8	92.0	286	34.1	3.1	1.7
0145	GG	N	GG	AA	N	TT	TT	GG	5.62	15.3	48.8	86.8	27.3	31.5	2.6	2.9
0232	GG	**FS**	GG	AA	N	TT	TT	GG	4.66	14.6	41.9	90.0	31.3	34.8	2.9	1.6
0529	GG	N	**GA**	AA	N	TC	TT	GG	5.32	15.8	45.1	84.9	29.6	34.9	3.0	1.8
0645	GG	N	**GA**	AA	N	TT	TT	GG	4.43	13.5	37.6	84.9	30.5	35.9	3.2	1.5
0676	GG	N	GG	AA	N	TC	TT	GG	4.28	12.2	33.5	78.3	28.5	36.4	3.0	1.7
0682	GG	N	GG	AA	N	TC	TT	GG	4.57	12.4	33.8	73.9	27.2	36.7	2.7	2.0
0687	GG	N	GG	AA	N	TT	TT	GG	4.95	15.0	41.3	83.5	30.3	36.3	2.9	1.8
0759	GG	N	GG	AA	N	TT	TT	GG	4.34	12.5	31.9	73.7	28.8	39.1	2.4	1.6
0766	GG	N	**GA**	AA	N	TT	TT	GG	4.92	14.8	37.7	76.6	30.1	39.3	2.5	2.0
0853	GG	N	**GA**	AA	N	**CC**	TT	GG	4.06	12.5	33.0	81.2	30.8	37.9	2.7	1.6
0873	GG	N	GG	AA	**FS**	TT	TT	GG	4.50	13.0	35.2	78.3	29.0	37.1	3.4	2.0
0886	GG	N	GG	AA	N	TC	TT	GG	5.20	13.3	37.3	71.7	25.7	35.8	3.0	2.1
0902	GG	N	GG	AA	N	TT	TT	GG	4.49	12.9	35.8	79.7	28.7	36.0	2.0	1.7
1072	GG	N	**GA**	AA	N	TT	TT	GG	4.42	13.4	35.7	80.8	30.3	37.5	2.8	2.5
1257	GGG	N	GG	AA	N	TT	TT	GG	4.83	13.9	37.3	77.3	28.7	37.2	2.4	1.6
1471	**GA**	N	GG	AA	N	TC	TT	GG	4.27	12.4	33.2	77.8	29.2	37.5	2.7	2.0
1475	GG	N	GG	AA	N	TT	TT	**GT**	4.93	13.1	36.5	74.1	26.6	35.9	2.8	1.9
1505	GG	N	GG	AA	N	TT	TT	GG	4.35	14.0	36.4	83.6	32.2	38.5	2.7	1.6
1518	GG	N	GG	AA	N	TT	TT	GG	4.44	13.5	36.0	81.0	30.5	37.7	3.0	1.5
1532	**GA**	N	GG	AA	N	TC	TT	GG	4.70	13.9	37.1	78.8	29.6	37.6	2.7	2.0
1620	GG	N	GG	AA	N	TT	TT	GG	4.50	12.6	33.5	74.4	28.0	37.7	3.1	1.6
1641	GG	N	GG	AA	N	TC	TT	GG	4.02	12.7	34.3	85.2	31.5	37.0	2.9	2.8
1645	GG	N	GG	AA	N	TT	TT	GG	4.57	13.1	35.2	77.1	28.6	37.2	2.7	2.0
1742	GG	N	**GA**	AA	N	**CC**	TT	GG	4.08	12.3	34.4	84.3	30.2	35.8	3.0	1.5
1744	GG	N	**GA**	AA	N	TC	TT	GG	4.93	14.9	41.8	84.7	30.2	35.6	3.2	2.4
1937	GG	N	GG	AA	N	TT	TT	GG	4.56	13.6	37.0	81.1	29.9	36.9	2.4	2.1
2245	GG	N	GG	AA	N	TT	TT	GG	4.45	11.4	34.5	77.6	25.6	33.0	3.5	2.4
2267	GG	N	GG	**AC**	N	TC	TT	GG	4.25	13.0	38.7	91.0	30.7	33.8	2.9	2.6
2321	GG	N	GG	AA	N	TT	TT	**GT**	4.58	13.2	36.8	80.3	28.8	35.9	3.1	3.5
2349	GG	N	*	AA	N	**CC**	TT	GG	4.51	13.7	37.0	81.9	30.4	37.1	3.4	1.7
2351	GG	N	GG	**AC**	N	TC	TT	GG	4.22	13.4	36.1	85.5	31.7	37.0	3.2	1.7
2396	GG	N	GG	AA	N	TT	TT	GG	4.35	13.1	35.9	82.6	30.1	36.5	2.8	3.8
2514	GG	N	**GA**	AA	N	TT	TT	GG	4.71	13.3	36.1	76.5	28.2	36.8	2.5	1.6
2606	GG	N	**GA**	AA	N	TT	TT	GG	4.81	15.2	42.5	88.2	31.5	35.7	2.4	2.2
2821	GG	N	**GA**	**AC**	N	TC	TT	GG	4.50	13.9	35.6	79.2	30.9	39.0	2.9	1.8
2824	GG	N	GG	**AC**	N	TC	TT	GG	4.91	14.9	38.9	79.2	30.4	38.4	3.0	1.7
2839	GG	N	GG	AA	N	TC	**TC**	GG	4.58	13.4	35.3	77.0	29.2	38.0	2.9	1.9
2933	GG	N	GG	AA	N	TC	TT	GG	4.28	12.8	33.9	79.3	29.9	37.6	2.8	1.5
3016	GG	N	GG	AA	N	TT	TT	GG	4.56	13.9	37.2	81.6	30.5	37.3	2.9	2.8
3154	GG	N	GG	**AC**	N	TC	TT	GG	5.20	16.3	41.8	80.4	31.3	38.9	2.8	2.6
3274	GG	N	GG	AA	N	TT	TT	GG	4.36	11.8	31.8	72.9	27.0	37.1	2.9	1.6
3326	GG	N	GG	AA	N	TT	TT	GG	4.73	14.6	38.4	81.1	30.9	38.2	2.6	3.1
3454	GG	N	GG	AA	N	TC	TT	GG	4.93	15.3	41.2	83.4	31.0	37.2	3.0	1.7
3590	GG	N	GG	AA	N	TC	TT	GG	4.25	11.7	33.6	78.9	27.5	34.8	2.8	1.9
3601	GG	N	GG	AA	N	TC	TT	GG	4.11	12.2	33.7	82.1	29.8	36.2	3.0	1.6
3970	GG	N	GG	AA	N	TT	TT	GG	4.20	11.7	32.3	76.8	27.8	36.2	2.6	2.5
4224	GG	N	GG	AA	N	TC	TT	GG	4.88	11.9	32.2	65.9	24.4	37.1	2.5	1.5
4276	GG	N	GG	AA	N	TC	**TC**	GG	5.29	14.8	40.4	76.4	28.0	36.7	3.4	1.5
4553	GG	N	**GA**	AA	N	TC	TT	GG	4.89	13.7	36.8	75.3	28.1	37.3	3.3	2.1

N, Normal base sequence; FS, Frameshift; *, have not been studied before

tion, may have increased HbF levels due to haplotype-effect of these variations (15). Furthermore, some recent studies have shown that KLF1 has dual action on the beta globe gene (i.e. a direct action on cis sequences and an indirect one via BCL) such that different mutations may effect one or other functional domains (27,28).

As it can be understood, KLF1 regulates the BCL11A gene to function as an on-off key of the globin genes, at the same time coordinates with the KLF2 gene to bind to the promotor of the Foxm1 gene in blood cells to maintain the survival of peripheral blood cells and to protect the amount of globin mRNA that is specific to erythrocytes. Any mutation in this gene can disrupt the genetic function and cause anemia as well as an increase in HbF levels. Most of the variations detected in the second exon of the KLF1 gene of 53 HbF-high volunteers in this study are neutral variations in which a nonpolar amino acid is converted to another nonpolar amino acid. Hence, no correlation was found between high HbF levels and neutral variations in these volunteers. Only at two points a polar-nonpolar amino acid change was found. Of them, at one point a significant drop in the high HbF levels was observed while the other was high near to the critical point. In fact, these findings indicate that function disrupting variations in the KLF1 gene could alter the HbF levels. However, in this study, the low number of high HbF volunteers prevented us from achieving the net result. If the number of volunteers is increased, it is thought that these variations in the KLF1 gene could be associated with high levels of HbF.

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Conflict of Interests
Authors declares that there is no conflict of interests.

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