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Original Article

Prevalence of ß-Thalassemia Mutations among Northeastern Iranian Population and their Impacts on Hematological Indices and Application of Prenatal Diagnosis, a Seven-Years Study

Mohammad Ehsan Jaripour[1#], Kourosh Hayatigolkhatmi[1#], Vahid Iranmanesh[1], Farhad Khadivi Zand[1], Zahra Badiei[2], Hamid Farhangi[2], Ali Ghasemi[2], Abdollah Banihashem[2], Reza Jafarzadeh Esfehani[3] and Ariane Sadr-Nabavi[1,3,4]*.

[1] Iranian Academic Center for Education, Culture and Research, (ACECR), Mashhad, Iran.
[2] Department of Pediatric Diseases, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
[3] Department of Medical Genetics, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
[4] Medical Genetics Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
[#] These authors contributed equally to this work.

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Abstract. ***Background and Objective:*** **ß-thalassemia results from a diverse range of mutations inside the hemoglobin subunit β (*HBB*) gene. In a study of β-thalassemia carriers and some of their at-risk fetuses in the Khorasan province of Iran, we aimed to recognize the most common mutations in the region. We also investigated a possible link between these mutations and some of the relevant hematological indices.**

Methods: **Amplification-refractory mutation system-PCR (ARMS-PCR) was used to detect the typical *HBB* mutations among 1593 individuals, suspected of having a mutated *HBB* allele from March/2011 to January/2018. Sanger sequencing of *HBB* had been performed, where ARMS-PCR was uninformative. In some cases, reverse dot blot was utilized. Analysis of variance was used to compare parametric variables.**

Results: **Among 1273 ß-thalassemia carriers, the prevalence of the mutations were reported as follows: IVS-I-5 (42.03%), IVS-II-1 (11.23%), codons 8/9 (4.79%), codon 44 (4.56%), codon 15 (3.53%), Los Angeles (2.91%), codon 5 (2.75%), IVS-I-110 (2.51%), -88 (2.20%) and other mutations were less than 2% of all of the reported mutations. 644 conceptions were subjected to prenatal diagnosis, using chorionic villus sampling. 118 cases were reported as normal. 352 cases were detected as carriers. 174 cases were diagnosed as affected. There was a significant difference in mean corpuscular volume and hemoglobin A2 levels between the nine most commonly reported mutation types ($p<0.001$).**

Conclusion: **This study makes a reliable guide for ß-thalassemia diagnosis in the region. The possibility of a correlation between *HBB* mutations and hematological indices opens a gate of future investigations.**

Keywords: ß-thalassemia, Mutation prevalence, Complete blood count, Prenatal diagnosis, Khorasan.

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Correspondence: Dr. Ariane Sadr-Nabavi. Assistant professor in medical genetics and head of the ACECR medical genetics center of Mashhad. Medical Genetics Department, School of Medicine, Mashhad University of Medical Sciences, Azadi Square, Mashhad, Iran. Tel: 00985138002226. Email: Sadrnabavia@mums.ac.ir

Introduction. ß-thalassemia refers to a highly common hereditary hematological disorder, caused by depletion or absence of the ß-globin synthesis. Regarding molecular aspects, this autosomal recessive disorder occurs following mutations which affect chromosome 11 in ß chain locus, responsible for coding a 146 amino acids polypeptide.[1,2] Diminution of ß chain production, in turn, results in excess of α-globin chains, accumulation of extra hemoglobin (Hb) inside the red blood cells (RBCs), destruction of mature RBCs in spleen (hemolysis) and bone marrow hyperplasia as a result of its inconclusive reparative efforts.[1,2] The broad spectrum of mutations in the β-globin gene brings a variety of phenotypically different features in patients, which enable clinicians to classify these disorders into different types. These subtypes are known as non-transfusion dependent (ND) and transfusion dependent (TD) ß-thalassemia (AKA: minor ß-thalassemia and major ß-thalassemia respectively).[1,2] Majority of these mutations are nucleotide substitutions, frameshifts, and small deletions. Large deletions are rarely involved in the development of ß-thalassemia.[1-11] These mutations affect synthesis of the ß-globin chain differently. Some of them bring a very mild reduction in the ß-globin production ($ß^{++}$ allele) while others may result in marked depletion ($ß^{+}$ allele) or complete absence ($ß^{o}$ allele) of the β-globin polypeptide.[1,2] ß-thalassemia, as discussed, can be presented with various severities and differences among patients' hematological indices, based on the responsible mutation type.[1-5]

Researches prove that ß-thalassemia is one of the most frequent genetic diseases worldwide, with a range of ethnically and geographically distributed mutations.[1,2] Moreover, the large number of carriers is a warning for the health system and an emphasis on the importance of preventing programs.[3-14] ß-globin genetic mutations distribute among every ethnic group. Identification of these mutations helps authorities for more accurate evaluations and more practical prevention programs.[3-14]

The purpose of this study was to recognize the most common mutations related to ß-thalassemia in the Khorasan province of Iran and to find the possible relation of these mutations with some of the relevant hematological indices. These indices are presented in complete blood count (CBC) and Hb electrophoresis tests (identifying HbA1, HbA2, and HbF). These indices include RBC, Hb, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH).

Material and Methods. *The region of study:* Khorasan province of Iran is the largest province of the country. The greater Khorasan consists of Razavi Khorasan, Northern Khorasan, and Southern Khorasan. 8,000,000 people are estimated to reside in the greater Khorasan. The study happened in the Razavi Khorasan with ~6,500,000 residents, within the academic center for education, culture, and research (ACECR) medical genetics laboratory. The ACECR laboratory has received most of the ß-thalassemia detection referrals over the Razavi Khorasan province and even some districts of the Northern and the Southern Khorasan. We estimate that the ACECR laboratory is responsible for ß-thalassemia detection among at least ~70% of the Razavi Khorasan's population, which is an estimation of 4,550,000 individuals. Estimations are based on the latest national census program and details provided by the local health center through personal communications.

Study subjects: The population investigated in this study comprises of 1593 individuals with Fars ethnicity, suspected of possessing a mutated allele for ß-thalassemia and referred to the ACECR diagnostic medical genetics laboratory for molecular diagnosis. Personal consent form to obtain the permission to use the patients' samples and the relevant data in the research performed by the ACECR was signed by every single individual of this study. The ACECR ethics committee, which functions under the regulations of the national medical ethics committee, approved these consent forms. Clinical criteria for suspicious ß-thalassemia was determined by the hypochromic microcytic anemia, including decreased MCV (<80 fL) and MCH (<27 pg/cell) and unusual findings in Hb electrophoresis, including elevated HbA2 (≥ 3.5 %) or HbF (≥ 1%) and other abnormally high Hb variants.[15] Peripheral blood (PB) samples from subjects were collected in EDTA containing tubes. Furthermore, chorionic villus samples (CVS) were collected from 644

conceptions, whose fetuses were at risk of inheriting two mutated alleles for hemoglobin subunit β (*HBB*) gene. Samples were collected by a neonatologist and sent to the ACECR medical genetics laboratory for ß-thalassemia prenatal diagnosis (PND). All samples (PB and CVS) were collected since March of 2011 up to January of 2018.

DNA extraction: DNA from selected individuals was extracted using standard salting out method as described by.[16] DNA from CVS was isolated using QiAmp® DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany) as described by the company instructions.

Mutation detection: For each amplification-refractory mutation system (ARMS-PCR) was used to detect ten common β-globin mutations: IVS-I-5, IVS-II-1, IVS-I-110, IVS-I-1, IVS-II-745, IVS-I-6, codon 30, codon 39, codon 819, and codon 16.[17] In cases, where none of the mentioned variants was detected; standard Sanger sequencing of the *HBB* had been performed to reveal the mutation. Supplementary table 1 shows primer sequences, micro-tube components and the thermal protocol used for the mentioned methods. Moreover, in some emergency cases, reverse dot blot (strip assay) was conducted using Thalassemia StripAssays® kit (ViennaLab, Vienna, Austria). For ß-thalassemia PND cases, we have routinely confirmed the result with two alternative molecular techniques. (See supplementary files)

Statistical analysis: Data was analyzed using the statistical package for social sciences (SPSS) version 22 (IBM Inc, Chicago, Il, USA). Continuous data were checked for normality using the Kolmogorov-Smirnov test. Means and standard deviations (SD) were used to describe continuous variables while frequency and percentage were used to describe categorical variables. Analysis of variance (ANOVA) was used to compare parametric variables, including RBC count, Hb, MCV, MCH, HbA2, and HbA1, between the mutation categories while the Kruskal-Wallis test was used to compare non-parametric variable (HbF) values between mutation groups. Multinomial logistic regression was performed to assess the relationship between the mutation categories and the study parameters considering other mutations than the nine evaluated (mostly reported mutations) as the reference category. The odds ratio (OR) and 95% confidence interval (CI) for OR were presented along with p-value for the regression model. Values of p less than 0.05 were considered statistically significant.

Results. As indicated in **figure 1**, among 1273 ß-thalassemia carriers studied over seven years (out of 1593 suspected referrals, whom 320 of them were not confirmed to have a pathogenic *HBB* variant based on our diagnostic molecular methods as discussed in the 'material and methods' section) the most commonly reported mutations were in a decreasing order of: IVS-I-5 (*HBB*: c.92+5G>C/A/T), IVS-II-1 (*HBB*: c.315+1G>A/C), codons 8/9 (*HBB*:c.27_28insG), codon 44 (*HBB*:c.135delC), codon 15 (*HBB*:c.48G>A), Los Angeles (*HBB*:c.364G>C), codon 5 (*HBB*:c.17_18delCT), IVS-I-110 (*HBB*:c.93-21G>A), -88 (*HBB*:c.-138C>A/G/T), IVS-I-6 (*HBB*:c.92+6T>C), codon 39 (*HBB*:c.118C>T), IVS-II-745 (*HBB*:c.316-106C>G), -29 (*HBB*:c.-79A>G), δβ (various large deletions), 25bp deletion (*HBB*:c.93-22_95del), HbS (*HBB*:c.20A>T) as all addressed by HbVar database.[18] The rest of the mutations were less than 1% all of the reported variants. During the years of this study, 644 conception cases on the formerly mentioned ß-thalassemia carrier couples were subjected to PND, using CVS samples as described in the "methods" section. For 118 cases which were reported to carry no detectable pathogenic variant and 352 cases which were detected as carriers (heterozygous with one pathogenic variant in *HBB*) and expected to show the ND ß-thalassemia phenotype, pregnancies were followed up till the delivery. Whereas 174 cases, which were diagnosed as homozygous with two pathogenic variants in *HBB* and expected as TD ß-thalassemia phenotype, have received the proper genetic counseling based on the regional guidelines and prevention programs.[13,14] Further, the relation of the first nine more commonly reported mutations with the hematological indices was observed as mentioned previously. There was a significant difference in MCV and HbA2 levels between the mutation types ($p<0.001$) (**Table 1**). Multinomial logistic regression model revealed that mutation categorized as IVS-II-I was associated with

increased risk for higher MCH (p=0.01, OR=1.15, 95% CI for OR= 1.03 and 1.28) and HbA2 (p=0.002, OR= 1.26, 95% CI for OR= 1.09, 1.46) and lower MCV (p<0.001, OR=0.93, 95% CI for OR= 0.90, 0.97) compared to other mutations. Furthermore, the codons 8/9 mutation was found to be associated with significant increase in HbF values compared to other mutations (p=0.04, OR= 1.05, 95% CI for OR= 1.00, 1.09).

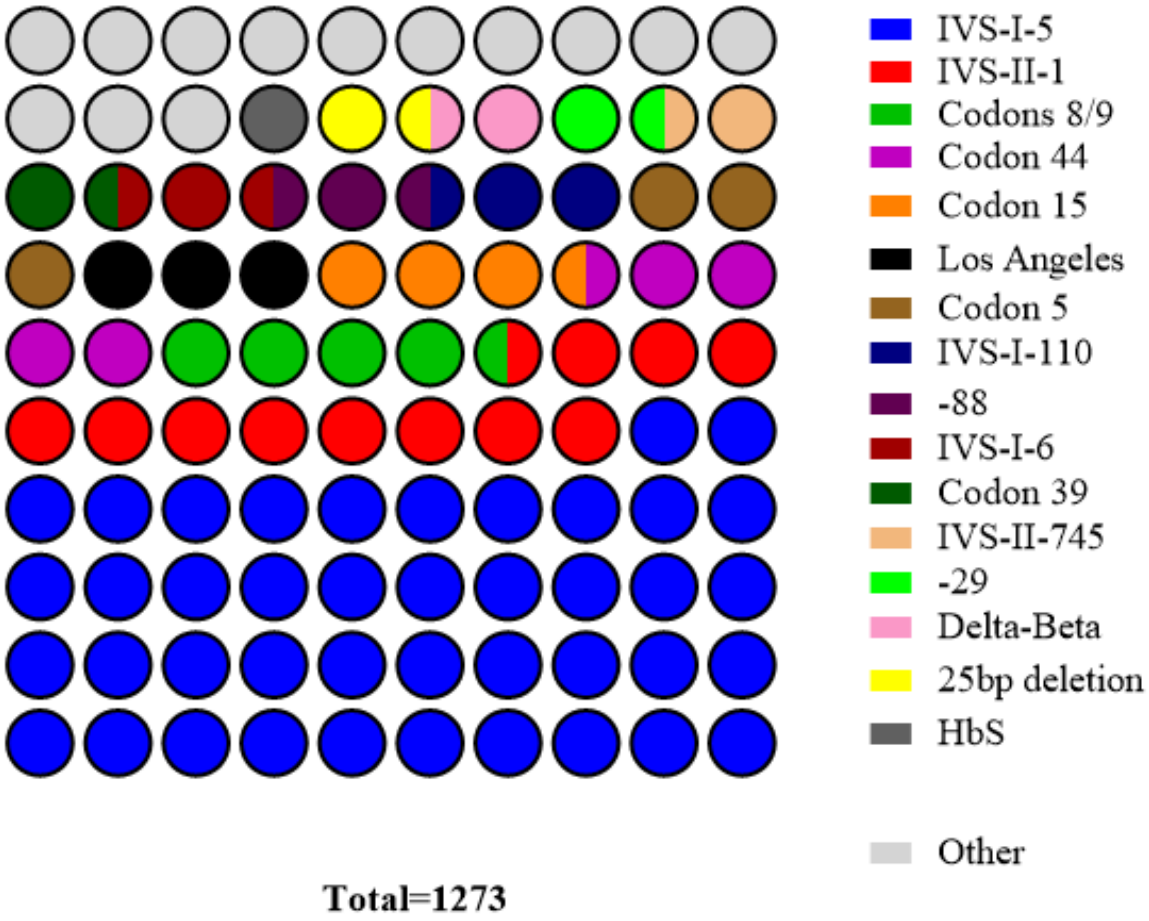

Figure 1. The 10 x 10 dot plot, showing the prevalence of ß-thalassemia mutations among 1273 ß-thalassemia carriers detected in the ACECR medical genetics laboratory of Mashhad, during 7 years. The ratios are as follows: IVS-I-5 (42.03%), IVS-II-1 (11.23%), codons 8/9 (4.79%), codon 44 (4.56%), codon 15 (3.53%), Los Angeles (2.91%), codon 5 (2.75%), IVS-I-110 (2.51%), -88 (2.20%), IVS-I-6 (1.81%), codon 39 (1.73%), IVS-II-745 (1.49%), -29 (1.49%), δβ (1.41%), 25bp deletion (1.41%), HbS (1.34%) and all other detected mutations consisted 12.80% of all of the reported pathogenic variants.

Indices/Mutation Unit/(Number)	***IVS-I-5 (535)***	***IVS-II-1 (143)***	***Codons 8/9 (61)***	***Codon 44 (58)***	***Codon 15 (45)***	***Los Angeles (37)***	***Codon 5 (35)***	***IVS-I-110 (32)***	***-88 (28)***	***p***
RBC x 10^6 cells/mcL ±SD	5.87 ± 0.48	5.84 ±0.79	5.85 ±0.78	5.67 ±0.65	5.75 ±0.83	5.89 ±0.86	5.54 ±0.75	5.46 ±0.86	5.57 ±1.23	0.30
Hb gm/dL ±SD	13.23 ±1.98	12.62 ±5.14	11.86 ±1.60	11.84 ±1.4	11.68 ±1.60	11.65 ±2.08	11.82 ±1.83	11.59 ±1.58	11.47 ±2.54	0.66
MCV fL ±SD	68.33 ±6.21*	64.59 ±8.71*	66.88 ±6.83*	68.62 ±6.97*	67.13 ±5.18	62.99 ±5.27	69.12 ±7.05*	68.61 ±5.95*	65.00 ±12.98	0.03*
MCH pg/cell ±SD	22.46 ±2.11	20.87 ±3.16	20.41 ±2.69	21.16 ±2.95	20.79 ±2.54	19.77 ±2.16	21.42 ± 2.67	21.58 ±3.61	20.07 ±4.20	0.47
HbA2 percent ±SD	4.56 ±1.59	4.72 ±1.42*	4.48 ±1.30*	3.80 ±1.35*	4.11 ±1.10	4.64 ±0.95	3.80 ±0.86	4.32 ±1.18	4.19 ±1.08*	0.003*
HbF percent ±SD	3.16 ±4.61	1.62 ±8.22	3.08 ±14.62	3.03 ±13.45	2.28 ±5.20	0.26 ±0.29	2.37 ±3.62	0.74 ±1.61	1.64 ±2.64	0.81ǂ

Table 1. Mean values of the relevant CBC indices and Hb subtypes among the studied population, carrying one of the first nine mostly common *HBB* pathogenic variants reported in the Khorasan province. The total number of studied individuals is indicated in parenthesis under mutations' names. Hb subtypes are reported as a percent of the total Hb amount of the individuals' PB sample. dL: deciliter; fL: femtoliter; gm: grams; mcL: microliter; p: p-value; pg: picogram. * Significant difference at α=0.05.ǂ The Kruskal Wallis test was used for the comparison while other parameters were compared between groups using the ANOVA test.

Discussion. Despite the spacious area and of course the massive population of the greater Khorasan, this province shows one of the lowest prevalence for ß-thalassemia in Iran.[6,7] Still, ß-thalassemia is one of the most hereditary hematological disorders reported routinely in this province.[6,7] Prior to the current study, there was almost no similar report on the ratio of ß-thalassemia mutations over this area, especially with such a vast pool of carriers. Just a previous study has named the codons 8/9 variant as the most prevalent mutation in the region.[6] Our research has contradicted this previous finding and identified the IVS-I-5 as the most frequent mutation, confirming the review by Mahdieh *et al*., 2016,[7] while the codons 8/9 is recognized as the third most common mutation. The ß-thalassemia mutations' prevalence reported by this study can act as a reliable resource for molecular diagnostic laboratories over the province, due to its considerable number of studied cases and the long time-period of observation. However, we have experienced some limitations, including the unwillingness of some of the potential carrier couples to enroll in genetic counseling sessions and undertaking the ß-thalassemia detection tests following clinical referrals. Generally, since the two variants of IVS-I-5 and IVS-II-1 consist over 50% of the total ß-thalassemia causing mutations in the province, it will be of vital importance for the local diagnostic laboratories to initiate their molecular diagnosis with the specific focus on these two variants.

Additionally, we investigated the relation of the first nine most commonly reported mutations with the hematological indices as mentioned formerly. The highest and the lowest RBC mean values were reported among Los Angeles and IVS-I-110 carriers respectively (ranges from 5.89 - 5.46 x 10^6 cells/mcL). The highest and the lowest Hb mean values were reported among IVS-I-5 and -88 carriers respectively (ranges from 13.23 – 11.47 mg/dL). The highest and the lowest MCV mean values were reported among codon 5 and IVS-II-1 carriers respectively (ranges from 69.12 – 64.59 fL). The highest and the lowest MCH mean values were reported among IVS-I-5 and Los Angeles carriers respectively (ranges from 22.46 – 19.77 pg/cell). The highest and the lowest HbA2 mean values were reported among IVS-II-1 and codon 5 carriers respectively (ranges from 4.72 – 3.80% of the total Hb). The highest and the lowest HbF mean values were reported among IVS-I-5 and Los Angeles carriers respectively (ranges from 3.16 – 0.26% of the total Hb). The highest and the lowest HbA1 mean values were reported among IVS-I-110 and IVS-I-5 carriers respectively (ranges from 94.92 – 83.26% of the total Hb). Hence, these reported values while considering the SD, p-value, OR and referring to the relevant guidelines can be helpful in offering a hint to the local clinicians for more accurate referrals. This can also be helpful as a boost for laboratory professionals for a more straightforward ß-thalassemia testing guide in the region. We have also found a significant difference regarding the MCV and the HbA2 levels between the mutation types. Our findings mean that the type of mutation causing ß-thalassemia has a high chance of affecting the MCV value and HbA2 ratio. In addition, moving further into the details of mutations impact on hematological indices we have illustrated that IVS-II-1 was associated with increased risk for higher MCH and HbA2 in comparison to other reported variants. It is also causing a lower MCV compared to other mutations. Also, the codons 8/9 mutation was found to be associated with significant increase in HbF values compared to other mutations. That means that the IVS-II-1 variant has a high chance of increasing the MCH and HbA2 while lowering the MCV when compared to other mutation types. On the other hand, the presence of the codons 8/9 will probably raise the HbF proportion when compared to other mutations. These findings act as a start point for more focused interdisciplinary studies on the genomic and hematologic profile of ß-thalassemia patients to find a more comprehensive map of genotype-phenotype correlation.

Conclusions. Results obtained from this study can help medical geneticists and other health care professionals in the province to detect the carriers and their at-risk fetuses through PND in a more rapid manner. Moreover, the idea of existing a logical correlation between pathogenic *HBB* variants and hematological indices can illuminate a new research topic of investigation for similar future studies. Also, the idea of how the mostly reported pathogenic variants might result in some genotype-phenotype correlation in the region might assist the relevant local clinicians for more rapid and accurate referrals to genetics services.

As previously discussed, ß-thalassemia is one of the most common genetic disorders worldwide and also in Iran.[3-14] Although there are some treatments available for controlling and recovering the disease such as routine blood transfusion (followed by the iron chelation therapy), bone marrow transplantation and even gene therapy, still genetic counseling and PND are known to be the best available preventive options.[1,2] Authors hope the current study will make a more accurate and useful guide for ß-thalassemia diagnosis and prevention in the region.

Acknowledgment. This study was performed on patients' recourses in the ACECR medical genetics laboratory of Mashhad as formerly addressed, based on medical ethics and privacy policy regulations. Authors are thankful to the medical genetics center of ACECR, the Mashhad branch, for their support over this study.

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Thalassemia PCR Program

IVS I.I

PCR Protocol

MASTER-MIX	*n=1*	*Cat-Nr*
H_2O	18.5 µl	G-1000-1 250 units
10x PCR Buffer	2.5 µl	G-1000-1 250 units
MgCl2 (25mM)	1 µl	G-1000-1 250 units
dNTP (10 mM)	0.5 µl	G-1000-1 250 units
Primer **IVS I.I** (1:10)	0.3 µl	BIO NEER
	0.3 µl	
Prime Taq DNA Polymerase (5 U/µl)	0.3 µl	G-1000-1 250 units
DNA	1 µl	

PCR Program:Thal 2

T°c	Time (s)	cycle
94	5min	1
94	40	30
59	40	
72	40	
72	5min	1

IVS I-5

PCR Protocol

MASTER-MIX	*n=1*	*Cat-Nr*
H_2O	17.5 µl	G-1000-1 250 units
10x PCR Buffer	2.5 µl	G-1000-1 250 units
MgCl2 (25mM)	2 µl	G-1000-1 250 units
dNTP (10 mM)	0.5 µl	G-1000-1 250 units
Primer **IVS I.5** (1:10)	0.3 µl	BIO NEER
	0.3 µl	
Prime Taq DNA Polymerase (5	0.3 µl	G-1000-1 250 units
DNA	1 µl	

PCR Program: Thal 1

T°c	Time (s)	cycle
94	5min	1
94	40	30
60	40	
72	40	
72	5min	1

IVS I.6

PCR Protocol

MASTER-MIX	*n=1*	*Cat-Nr*
H_2O	17.5 µl	G-1000-1 250 units
10x PCR Buffer	2.5 µl	G-1000-1 250 units
MgCl2 (25mM)	2 µl	G-1000-1 250 units
dNTP (10 mM)	0.5 µl	G-1000-1 250 units
Primer **IVS I.6** (1:10)	0.3 µl	BIO NEER
[illegible]	0.3 µl	
Prime Taq DNA Polymerase (5	0.3 µl	G-1000-1 250 units
DNA	1 µl	

PCR Program: Thal 1

T°c	Time (s)	cycle
94	5min	1
94	40	30
60	40	
72	40	
72	5min	1

Codon30

PCR Protocol

MASTER-MIX	*n=1*	*Cat-Nr*
H_2O	17.5 µl	G-1000-1 250 units
10x PCR Buffer	2.5 µl	G-1000-1 250 units
MgCl2 (25mM)	2 µl	G-1000-1 250 units
dNTP (10 mM)	0.5 µl	G-1000-1 250 units
Primer **C30** (1:10)	0.3 µl	BIO NEER
[illegible]	0.3 µl	
Prime Taq DNA Polymerase (5	0.3 µl	G-1000-1 250 units
DNA	1 µl	

PCR Program: Thal 3

T°c	Time (s)	cycle
94	5min	1
94	40	28
	40	
72	40	
72	5min	1

IVS I.110

PCR Protocol

MASTER-MIX	*n=1*	*Cat-Nr*
H_2O	17.5 µl	G-1000-1 250 units
10x PCR Buffer	2.5 µl	G-1000-1 250 units
MgCl2 (25mM)	2 µl	G-1000-1 250 units
dNTP (10 mM)	0.5 µl	G-1000-1 250 units
Primer **IVS I.110** (1:10)	0.3 µl	BIO NEER
[illegible]	0.3 µl	
Prime Taq DNA Polymerase (5	0.3 µl	G-1000-1 250 units
DNA	1 µl	

PCR Program: Thal 1

T°c	Time (s)	cycle
94	5min	1
94	40	30
60	40	
72	40	
72	5min	1

Codon39

PCR Protocol

MASTER-MIX	*n=1*	*Cat-Nr*
H_2O	17.5 µl	G-1000-1 250 units
10x PCR Buffer	2.5 µl	G-1000-1 250 units
MgCl2 (25mM)	2 µl	G-1000-1 250 units
dNTP (10 mM)	0.5 µl	G-1000-1 250 units
Primer **C39** (1:10)	0.3 µl	BIO NEER
[illegible]	0.3 µl	
Prime Taq DNA Polymerase (5	0.3 µl	G-1000-1 250 units
DNA	1 µl	

PCR Program: Thal 1

T°c	Time (s)	cycle
94	5min	1
94	40	30
60	40	
72	40	
72	5min	1

Fr16

PCR Protocol

MASTER-MIX	*n=1*	*Cat-Nr*
H_2O	17.5 µl	G-1000-1 250 units
10x PCR Buffer	2.5 µl	G-1000-1 250 units
MgCl2 (25mM)	2 µl	G-1000-1 250 units
dNTP (10 mM)	0.5 µl	G-1000-1 250 units
Primer **Fr 16** (1:10)	0.3 µl	BIO NEER
	0.3 µl	
Prime Taq DNA Polymerase (5	0.3 µl	G-1000-1 250 units
DNA	1 µl	

PCR Program: Thal 1

T°c	**Time (s)**	**cycle**
94	5min	1
94	40	30
60	40	
72	40	
72	5min	1

Fr 82/83(new primer)

PCR Protocol

MASTER-MIX	*n=1*	*Cat-Nr*
H_2O	17.5 µl	G-1000-1 250 units
10x PCR Buffer	2.5 µl	G-1000-1 250 units
MgCl2 (25mM)	2 µl	G-1000-1 250 units
dNTP (10 mM)	0.5 µl	G-1000-1 250 units
Primer **Fr 82/83** (1:10)	0.3 µl	BIO NEER
	0.3 µl	
Prime Taq DNA Polymerase (5	0.3 µl	G-1000-1 250 units
DNA	1 µl	

PCR Program: Thal 1

T°c	**Time (s)**	**cycle**
94	5min	1
94	40	30
60	40	
72	40	
72	5min	1

IVS II.745 (new primer)

PCR Protocol

MASTER-MIX	*n=1*	*Cat-Nr*
H_2O	17.5 µl	G-1000-1 250 units
10x PCR Buffer	2.5 µl	G-1000-1 250 units
MgCl2 (25mM)	2 µl	G-1000-1 250 units
dNTP (10 mM)	0.5 µl	G-1000-1 250 units
Primer **IVS II.745** (1:10)	0.3 µl	BIO NEER
	0.3 µl	
Prime Taq DNA Polymerase (5	0.3 µl	G-1000-1 250 units
DNA	1 µl	

PCR Program: Thal 1

T°c	Time (s)	cycle
94	5min	1
94	40	30
60	40	
72	40	
72	5min	1

IVS II.I (new primer)

PCR Protocol

MASTER-MIX	*n=1*	*Cat-Nr*
H_2O	17.5 µl	G-1000-1 250 units
10x PCR Buffer	2.5 µl	G-1000-1 250 units
MgCl2 (25mM)	2 µl	G-1000-1 250 units
dNTP (10 mM)	0.5 µl	G-1000-1 250 units
Primer **IVS II-I** (1:10)	0.3 µl	BIO NEER
	0.3 µl	
Prime Taq DNA Polymerase (5	0.3 µl	G-1000-1 250 units
DNA	1 µl	

PCR Program: Thal 4

T°c	Time (s)	cycle
94	5min	1
94	40	30
62	40	
72	40	
72	5min	1

Fr 8/9

PCR Protocol

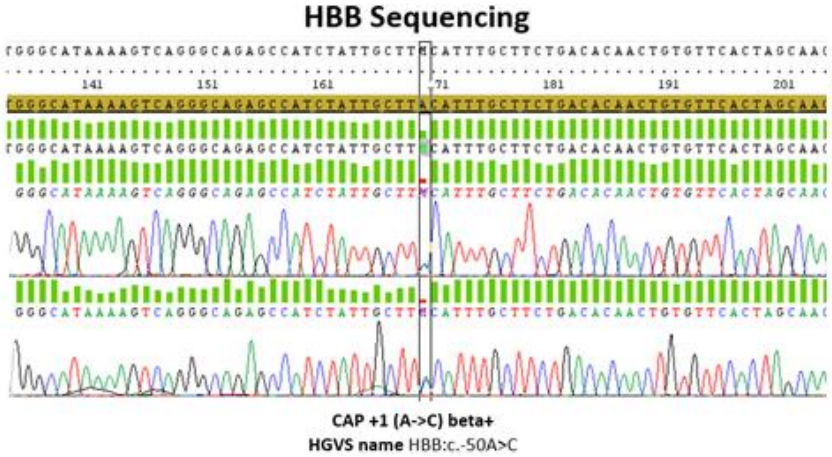
MASTER-MIX	*n=1*	*Cat-Nr*
H_2O	17.5 µl	G-1000-1 250 units
10x PCR Buffer	2.5 µl	G-1000-1 250 units
MgCl2 (25mM)	2 µl	G-1000-1 250 units
dNTP (10 mM)	0.5 µl	G-1000-1 250 units
Primer **Fr 8/9** (1:10)	0.3 µl	BIO NEER
[illegible]	0.3 µl	
Prime Taq DNA Polymerase (5	0.3 µl	G-1000-1 250 units
DNA	1 µl	

PCR Program: Thal 4

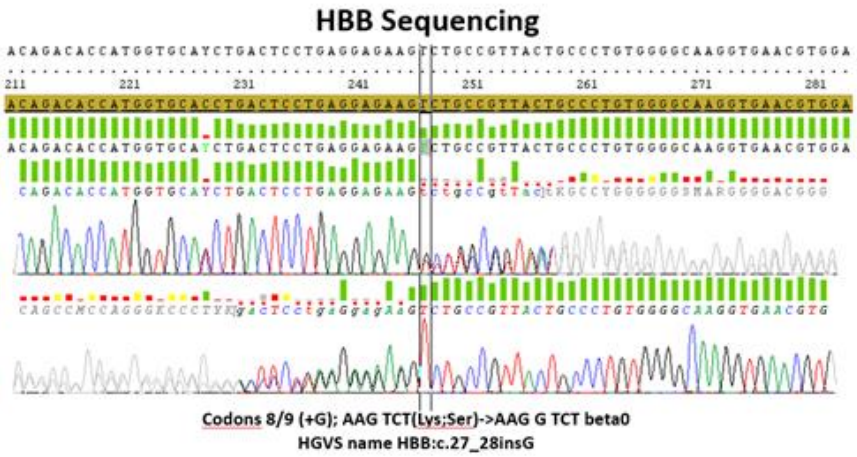
T°c	Time (s)	cycle
94	5min	1
94	40	30
62	40	
72	40	
72	5min	1

Primer sequences: 5'>3'

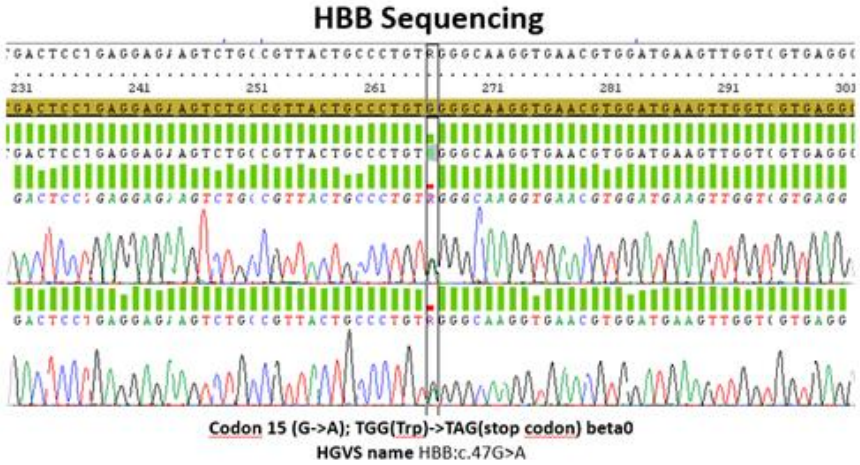
comB	**GACTCAAGGCTGAGAGATGCAGGA**
comA	**CAATGTATCATGCCTCTTTGCACC**
comC	**ACCTCACCCTGTGGAGCCAC**
IVS1.1M	**TTAAACCTGTCTTGTAACCTTGATACCCAT**
IVS1.1N	**TTAAACCTGTCTTGTAACCTTGATACCCAC**
IVS1.5M	**CTCCTTAAACCTGTCTTGTAACCTTGTTAG**
IVS1.5N	**CTCCTTAAACCTGTCTTGTAACCTTGTTAC**
IVS1.6M	**TCTCCTTAAACCTGTCTTGTAACCTTCATG**
IVS1.6N	**TCTCCTTAAACCTGTCTTGTAACCTTCATA**
IVS1.110M	**ACCAGCAGCCTAAGGGTGGGAAAATAGAGT**
IVS1.110N	**ACCAGCAGCCTAAGGGTGGGAAAATAGAGC**
IVS11.745 M	**GGTTTCATATTGCTAATAGCAGCTACAATCGAGG**
IVS11.745 N	**GGTTTCATATTGCTAATAGCAGCTACAATCGAGC**
C30 M	**TAAACCTGTCTTGTAACCTTGATACCAACG**
C30 N	**TAAACCTGTCTTGTAACCTTGATACCAACC**
C39 M	**CAGATCCCCAAAGGACTCAAAGAACCTGTA**
C39 N	**CAGATCCCCAAAGGACTCAAAGAACCTGTA**
fr16 N	**TCACCACCAACTTCATCCACGTTCACGTTG**
fr16 M	**TCACCACCAACTTCATCCACGTTCACGTTC**
IVS11.1 M	**AAGAAAACATCAAGGGTCCCATAGACTGAT**
IVS11.1 N	**AAGAAAACATCAAGGGTCCCATAGACTGAC**
fr8-9 M	**CCTTGCCCCACAGGGCAGTAACGGCACACC**
fr8-9 N	**CCTTGCCCCACAGGGCAGTAACGGCACACT**



CAP +1 (A->C) beta+
HGVS name HBB:c.-50A>C



Codons 8/9 (+G); AAG TCT(Lys;Ser)->AAG G TCT beta0
HGVS name HBB:c.27_28insG



Codon 15 (G->A); TGG(Trp)->TAG(stop codon) beta0
HGVS name HBB:c.47G>A

1 : minor beta thalassemia
2: minor beta thalassemia
3: normal
4: normal
5: major beta thalassemia (control)
6: minor beta thalassemia (control)
7: normal control
8: negative control