Genetic modifiers of β-thalassemia and clinical severity as assessed by age at first transfusion

Fabrice Danjou,[1]* Franco Anni,[1]* Lucia Perseu,[2] Stefania Satta,[1] Carlo Dessì,[1] Maria Eliana Lai,[3] Paolo Fortina,[4,5] Marcella Devoto,[5,6,7] and Renzo Galanello[1]

[1]Clinica Pediatrica 2a, Dipartimento di Scienze Biomediche e Biotecnologie - Università di Cagliari, Ospedale Regionale Microcitemie ASL8, Cagliari, Italy; [2]Istituto di Ricerca Genetica e Biomedica (IRGB) CNR, Cagliari, Italy; [3]Ospedale Regionale Microcitemie, ASL8 Cagliari, Italy; [4]Department of Cancer Biology, Jefferson Genomics Laboratory, Kimmel Cancer Center, Thomas Jefferson University Jefferson Medical College, Philadelphia, PA, USA; [5]Dipartimento di Medicina Molecolare, Università La Sapienza, Roma, Italy; [6]Division of Genetics, The Children's Hospital of Philadelphia, Philadelphia, PA, USA, and [7]Department of Pediatrics and Department of Biostatistics and Epidemiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

ABSTRACT

Background

The clinical and hematologic features of β-thalassemia are modulated by different factors, resulting in a wide range of clinical severity. The main factors are the type of disease-causing mutation and the ability to produce α-globin and γ-globin chains. In the present study we investigated the respective contributions of known modifiers to the prediction of the clinical severity of β-thalassemia as assessed by the patients' age at first transfusion.

Design and Methods

We studied the effect of seven loci in a cohort of 316 Sardinian patients with β^0-thalassemia. In addition to characterizing the β-globin gene mutations, α-globin gene defects and HBG2:g.-158C>T polymorphism, we genotyped two different markers in the *BCL11A* gene and three in the *HBS1L-MYB* intergenic region using single nucleotide polymorphism microarrays, imputation and direct genotyping. We performed Cox proportional hazard analysis of the time to first transfusion.

Results

According to the resulting model, we were able to explain phenotypic severity to a large extent (Harrell's concordance index=0.72; Cox & Snell R^2=0.394) and demonstrated that most of the model's discriminatory ability is attributable to the genetic variants affecting fetal hemoglobin production (HBG2:g.-158C>T, *BCL11A* and *HBS1L-MYB* loci: C-index=0.68, R^2=0.272), while the remaining is due to α-globin gene defects and gender. Consequently, significantly distinct survival curves can be described in our population.

Conclusions

This detailed analysis clarifies the impact of genetic modifiers on the clinical severity of the disease, measured by time to first transfusion, by determining their relative contributions in a homogeneous cohort of β^0-thalassemia patients. It may also support clinical decisions regarding the beginning of transfusion therapy in patients with β-thalassemia.

Key words: beta-thalassemia, genetic modifiers, fetal hemoglobin, thalassemia major, thalassemia intermedia.

Citation: Danjou F, Anni F, Perseu L, Satta S, Dessì C, Lai ME, Fortina P, Devoto M, and Galanello R. Genetic modifiers of β-thalassemia and clinical severity as assessed by age at first transfusion. Haematologica 2012;97(7):989-993. doi:10.3324/haematol.2011.053504

**These two authors contributed equally to this work*

Acknowledgments: we thank Serena Sanna for her support and constructive comments and Francarosa Demartis for her technical assistance.

Funding: supported by grants from Regione Autonoma Sardegna L.R. 11/90 and a Research Grant from Apopharma (Ontario – Canada).

Manuscript received on August 11, 2011. Revised version arrived on January 5, 2012. Manuscript accepted January 10, 2012.

Correspondence:
Renzo Galanello, Ospedale Regionale Microcitemie, Via Jenner s/n, 09121 Cagliari, Italy.
Phone: international + 39.070.6095508.
Fax: international + 39.070.6095509.
E-mail: renzo.galanello@mcweb.unica.it

Introduction

β-thalassemia is characterized by decreased or absent β-globin chain synthesis due to a variety of mutations; this decrease in β-globin chain synthesis results in an excess of α-globin chains which precipitate in red blood cell precursors in the bone marrow, causing their premature death.[1] In Sardinia, the most common type of β-thalassemia is due to a nonsense mutation at codon 39 of the β-globin gene (HBB:c118C>T).[2]

The majority of patients develop a severe form of anemia (thalassemia major) and are transfusion-dependent from the first years of life. When performed regularly, red blood cell transfusions prevent anemia-related complications and compensatory marrow expansion, and, therefore, extend the survival of patients. Approximately 5-10% of patients live without requiring periodic blood transfusions and are said to have thalassemia intermedia.[3] These two forms of the disease are the extreme ends of a broad range of clinical variability: patients might need to start transfusions after days, months or even years of life, demonstration of a great variation in disease severity.

This remarkable phenotypic diversity of thalassemia patients is associated with a great variety of genotypes including mild/silent β-thalassemia alleles, coinheritance of α-thalassemia or the presence of genetic determinants associated with increased production of γ-globin chains and consequent ability to produce functional fetal hemoglobin (Hb F) in adult life.[4] All these conditions reduce α/β-globin chain imbalance and ineffective erythropoiesis. The level of Hb F is regulated by three major loci: HBG2:g.-158C>T on 11p15.4, *HBS1L-MYB* intergenic region on 6q23.3, and *BCL11A* on 2p16.1. Together these three loci are responsible for 20 to 50% of the Hb F trait variance in patients with β-thalassemia or sickle cell disease, and in healthy Europeans.[5-10]

Here we evaluated the effect of the HBG2:g.-158C>T, *BCL11A*, and *HBS1L-MYB* variants, together with coinheritance of α-thalassemia and gender, on the severity of β-thalassemia phenotype, measured by age at first transfusion, in Sardinian patients.

Design and Methods

Patients and phenotypic assessment

We retrospectively studied 316 β^0-thalassemia patients (168 males and 148 females) from Sardinia, all followed at the Microcythemia Hospital of Cagliari. Of these patients, 266 had thalassemia major (median age 33 years; 5th and 95th percentiles, 13 and 38 years, respectively) and 50 had thalassemia intermedia (median age 43 years; 5th and 95th percentiles, 17 and 61 years, respectively); 125 had been enrolled in a previous study on phenotype amelioration.[11] Thalassemia intermedia patients were defined as patients who had never been transfused, or had only been transfused sporadically during infections or surgery (<10 blood units in total).[3] The β-thalassemia mutations were of HBB:c118C>T/HBB:c118C>T type in 92.4% of cases and HBB:c118C>T/HBB:c.20delA type in 6.3% of the studied sample; the remaining mutations are reported in Table 1. The continuous distribution of the phenotypic severity among thalassemia patients was measured by the time at which they started transfusion therapy. Criteria for starting transfusion were persistent (i.e. more than 2 weeks) hemoglobin level lower than 7 g/dL in the absence of infections, moderate to severe spleen enlargement and poor growth. The time to event was calculated as the time between birth and the first red blood cell transfusion or between birth and the last follow-up (January 2011) for patients who were not on transfusion therapy. Age at first transfusion was retrospectively collected through the WebTHAL computerized clinical records database (*http://www.thalassemia.it*), in use for the daily management of patients in our center.

This retrospective study was conducted in accordance with the Declaration of Helsinki and the patients gave informed consent to analysis of their DNA.

Selection of single nucleotide polymorphisms

We selected five single nucleotide polymorphisms (SNP) from the *HBS1L-MYB* intergenic region and the *BCL11A* locus known to be associated with Hb F levels (Table 2):

rs1427407: the most significant SNP associated with Hb F levels within *BCL11A*, as reported by Menzel *et al.*[12] This SNP is in high linkage disequilibrium (LD) with rs766432 (r^2=0.98) in our sample, and with rs4671393 (r^2=0.88 / D'=1) in the CEU samples based on the 1000 Genomes Project pilot phase 1 (CEU.1kG), for which effects on Hb F levels are also well-documented;[13,14]

rs10189857: within *BCL11A*, documented to have an independent effect on Hb F levels;[15]

rs9399137: the most significant SNP for Hb F levels within the *HBS1L-MYB* intergenic region in different populations,[5,12] in complete LD with a 3-bp deletion located in close proximity to four erythropoiesis-related transcription factor binding sites;[15,16]

rs4895441: a SNP within the *HBS1L-MYB* intergenic region, widely reported to be associated with Hb F levels[5,17] and in complete LD with rs9402686 (r^2=1 / D'=1 from CEU.1kG data), also reported to be independently associated with Hb F levels;[15]

rs6904897: within the *HBS1L-MYB* intergenic region, this SNP is in complete LD with rs28384513 (r^2=1 / D'=1 from CEU.1kG data), reported to be independently associated with Hb F levels.[15]

Genotyping

DNA was extracted from venous peripheral blood with standard methods. Mutations of the β-globin gene were analyzed by direct DNA sequencing. The HBG2:g.-158C>T polymorphism was determined as described elsewhere.[18] α-globin gene defects were determined using gap-polymerase chain reaction or restriction enzyme digestion for deletional and non-deletional defects, respectively.[19]

SNP were directly genotyped except for rs4895441 which was genotyped using the Affymetrix Genome-Wide Human SNP Array 6.0 according to the manufacturer's protocol and rs6904897 which was imputed with MACH software, version 1.0.16, using a combined panel of Utah Residents of Northern and Western European Ancestry (CEU) and Tuscan samples (TSI) from the International Hapmap consortium as reference samples.[20]

Sixteen samples (from patients selected for being positive for the HBG2:g.-158C>T polymorphism) for which SNP array data were not available, were genotyped using TaqMan SNP genotyping assay (Applied Biosystems, Warrington, UK) for each of the five SNP.

Quality controls

Microarray data from the samples underwent quality control procedures, including: sample call rate (exclusion when the call rate was <95%), cryptic relatedness (exclusion of first degree relatives), inbreeding coefficient (exclusion if negative with a call rate <98%) and reported gender *versus* heterozygosity of X chromosome SNP (exclusion if discordant). Principal component analysis, as implemented in EIGENSTRAT, was performed for the detection of outliers.[21] Quality control attributes for the SNP used in the present study are described in Table 2.

Statistical analysis

All genome-wide quality control measures were performed using the PLINK software package, version 1.07,[22] while the SPSS statistical software package, version 18.00 (SPSS, IBM, Somers, NY, USA), was used for subsequent analysis. All markers selected for the present study were entered in a backward stepwise Cox proportional hazard model to characterize their effect on time to first transfusion, together with gender, α-globin gene defects and status for HBG2:g.-158C>T polymorphism (only -/- and +/- genotypes were observed for this SNP). For each SNP a variable was defined with the value of 0, 1, or 2 according to the number of copies of the less frequent allele, except for rs6904897: since there was no difference between the G/G and G/T genotype survival curves for this SNP, it was codified 0 for both these genotypes and 1 otherwise. α-globin gene defects were classified as 0, 1, or 2 according to the number of deleted or mutated copies of the *HBA* gene (see Table 1 for details). Gender was codified 0 when female and 1 when male. Covariates were excluded from the model when their *P* value was greater than 0.10. Patients were considered uncensored when blood transfusion occurred during the study and censored when blood transfusion did not occur. We report Cox and Snell R^2 as well as Harrell's concordance index (C-index) to assess how well the model performed.

Table 1. Genotypic frequencies of genetic markers and clinical characteristics.

			Cases (%)	Median time to first transfusion in months (5th-95th percentile)	Thalassemia intermedia patients (% per row)[1]
β^0 Genotype		HBB:c118C>T/HBB:c118C>T	292 (92.4)	9 (3-53)	13.7
		HBB:c118C>T/HBB:c.20delA	20 (6.3)	32 (8-83)	50.0[2]
		HBB:c118C>T/HBB:c.230delC	3 (0.9)	18 (14-91)	0.0
		HBB:c118C>T/HBB:c.315+1G>A	1 (0.3)	7 (7-7)	0.0
HBG2:g.-158C>T	-/-	300 (94.9)	9 (3-57)	12.3	
	+/-	16 (5.1)	13 (9-63)	81.3	
α gene defects[3]					
	class 0	αα/αα	169 (53.5)	7 (2-49)	7.1
	class 1	-α/αα	94 (29.7)	11 (3-50)	21.8
		α^{NcoI}α/αα	12 (3.8)		
		α^{HphI}α/αα	2 (0.6)		
		$-\alpha^{4.2}$/αα	2 (0.6)		
	class 2	-α/-α	30 (9.5)	34 (8-80)	37.8
		-α/α^{NcoI}α	5 (1.6)		
		-α/α^{HphI}α	1 (0.3)		
		$-\alpha^{3.7}/-\alpha^{4.2}$	1 (0.3)		
BCL11A	rs1427407	T/T	7 (2.2)	11 (10-25)	42.9
		G/T	93 (29.4)	16 (3-77)	28.0
		G/G	216 (68.4)	7 (2-57)	9.7
	rs10189857	G/G	50 (15.8)	12 (4-57)	14.0
		A/G	154 (48.7)	9 (3-66)	13.0
		A/A	112 (35.4)	8 (2-49)	20.5
HBS1L-MYB intergenic region	rs9399137	C/C	6 (1.9)	28 (3-32)	50.0
		T/C	100 (31.6)	12 (3-86)	20.0
		T/T	210 (66.5)	9 (3-49)	12.9
	rs4895441	G/G	17 (5.4)	9 (3-80)	29.4
		G/A	106 (33.5)	14 (3-63)	18.9
		A/A	193 (61.1)	8 (2-49)	13.0
	rs6904897	G/G	22 (7)	9 (3-63)	27.3
		G/T	98 (31)	10 (2-57)	16.3
		T/T	196 (62)	10 (3-57)	14.3

[1]overall 15.8% of patients had the intermedia form of the disease (50/316). [2]55% of HBB:c118C>T/HBB:c.20delA patients are +/- for the HBG2:g.-158C>T polymorphism. [3]α^{HphI} refers to the HBA2:c.95+2_95+6delTGAGG whereas α^{NcoI} refers to the HBA2:c.2T>C mutation; $-\alpha^{3.7}$ and $-\alpha^{4.2}$ refer to the commonly denominated 3.7-kb rightward deletion and 4.2-kb leftward deletion of the α gene.

Table 2. Characteristics of single nucleotide polymorphisms used in the study.

Locus	SNP	Chromosome	Position (GRCh37)	Call rate / r^2	*P* value for Hardy-Weinberg equilibrium test	Minor allele frequency
BCL11A	rs1427407	2	60718043	CR=1.00 1	0.41	0.17
	rs10189857	2	60713235	CR=1.00 1	0.81	0.40
HBS1L-MYB intergenic region	rs9399137	6	135419018	CR=1.00 1	0.13	0.18
	rs4895441	6	135426573	CR=1.00	0.63	0.22
	rs6904897	6	135382980	r^2=0.99 2	0.05	0.22

[1]direct genotyping; [2]squared correlation between imputed and true genotypes.

Results

Results from the stepwise Cox proportional hazard model are presented in Table 3. We refer below to predictors for later time to transfusion as positive values and predictors for earlier time to transfusion as negative values.

The HBG2:g.-158C>T polymorphism had the strongest effect on the severity of β-thalassemia phenotype [hazard ratio (HR)=0.08; *P*<0.001], followed by rs1427407 (*BCL11A*) (HR=2.37; P<0.001), α-globin gene defects (HR=0.52; *P*<0.001), rs4895441 (*HBS1L-MYB*) (HR=1.94; *P*<0.001), rs10189857 (*BCL11A*) (HR=1.31; *P*=0.004), rs6904897 (*HBS1L-MYB*) (HR=0.79, *P*=0.047) and gender (HR=0.73; *P*=0.013). The SNP rs9399137 (*HBS1L-MYB*), in high LD with rs4895441 (r^2=0.90 from CEU.1kG data), was the only predictor removed from the model (HR=1.29; *P*=0.298). Among all two-way interactions tested, the only significant one was between rs1427407 and rs10189857 (HR=1.66; *P*=0.036).

The discriminatory power of the model was high (C-index=0.72; R^2=0.394) and most of it was attributable to Hb F production modulators (HBG2:g.-158C>T, *BCL11A* and *HBS1L-MYB* loci: C-index=0.68, R^2=0.272), while the remaining was attributable to α-globin gene defects and gender.

According to our model prediction, 50% of patients with all negative predictors would undergo their first transfusion within the first 100 days of life and 99% of them would need regular transfusions before the first year of life. On the other hand, with all positive predictors, the probability of undergoing transfusion by 10 years was only 6‰.

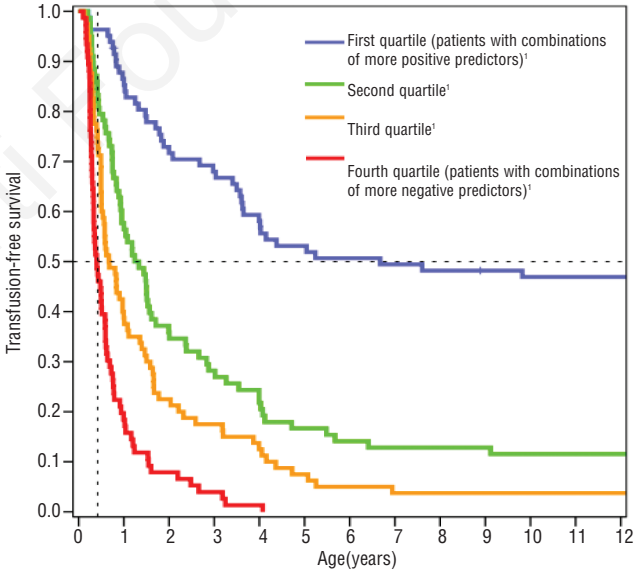
We evaluated survival curves for time to first transfusion for four groups defined by the quartiles of the distribution of the linear predictor score (i.e. the sum of the product between covariate values and their corresponding parameter estimates). Lower values (first quartile) corresponded to different combinations of mostly positive predictors (82 cases - linear predictor score values below 1.45), while higher values (fourth quartile) corresponded to different combinations of mostly negative predictors (76 cases - linear predictor score values above 2.70). Intermediate groups included 78 cases with linear predictor score values between 1.45 and 2.05 (second quartile) and 80 cases with linear predictor score values between 2.05 and 2.70 (third quartile).

Following this classification, 50% of patients in the fourth quartile group underwent their first transfusion within 6 months of life, whereas only 3% of patients in the first quartile group had started transfusions by the same age. In this group it took more than 6 years for 50% of the patients to start transfusions, whereas by the same age all patients in the fourth quartile group had undergone their first transfusion. In the first quartile group, 47% of patients never started red blood cell transfusion (Figure 1).

All survival curves were significantly different from each other (*P*<0.01, Breslow's test). In particular, the third quartile group was significantly different from the fourth quartile group (*P*<0.001) and the second quartile group was significantly different from both the first and third quartile risk-groups (*P*<0.001 and *P*=0.007, respectively).

Discussion

The purpose of this study was to measure the influence of known genetic modifiers of β^0-thalassemia on phenotype severity, assessed as time to first transfusion. To this aim, SNP in the *BCL11A* gene and *HBS1L-MYB* intergenic region were selected based on previous studies and genotyped in a group of 316 patients, as were α-globin gene defects and the HBG2:g.-158C>T polymorphism. All these variables, together with gender, were included in a Cox proportional



[1]Statistical tests of differences between the curves are reported in the text.

Figure 1. Kaplan-Meier survival curves for patients with different combinations of predictors for later or earlier transfusion need.

Table 3. Results of the Cox proportional hazards model.

Locus		*P*	Hazard ratio	Harrell's C-index	Predictor for later transfusion start
HBG2:g.-58C>T		<0.001	0.081	0.54	+/-
α gene defects		<0.001	0.514	0.61	class 2 1
BCL11A	rs1427407	<0.001	2.391	0.63	T allele
	rs10189857	0.005	1.312		G allele
HBS1L/MYB	rs4895441	<0.001	1.979	0.57	G allele
	rs6904897	0.020	0.697		TT genotype
Gender		0.016	0.738	0.52	Male

[1]*Definitions of classes are reported in Table 1.*

hazard model for time to first transfusion, and their respective effects were measured. The results showed that Hb F production variants and α-globin gene defects had a substantial impact on the severity of β-thalassemia phenotype, allowing prediction of the risk of patients to start transfusion at different times of their life.

In this study we assumed that the time to first transfusion accurately reflects variations in β-thalassemia phenotype severity. This hypothesis seems to be supported by our results, as all variables and the hierarchy of their effects agree with previous studies on genetic modifiers of both Hb F levels and the clinical severity of β-thalassemia, even though other unknown genetic factors and clinical conditions might be co-responsible for the need for transfusions.[3,11,15,23,24]

To the best of our knowledge, the present study is the first to analyze the severity of β-thalassemia in a quantitatively defined manner and to include such a complete set of known predictors. In a previous study, Galanello *et al.*[11] studied the effect of two SNP (rs11886868 in *BCL11A* and rs9389268 in the *HBS1L-MYB* intergenic region) and α-globin gene defects on the phenotypic expression (defined as major *versus* intermedia status) of Sardinian patients with β^0-thalassemia. A recent study by Badens *et al.*[24] further extended this analysis accounting for the HBG2:g.-158C>T polymorphism and β^0/β^+ status, in addition to the previously mentioned markers, in a heterogeneous cohort of 106 patients with 30 different β-globin gene mutations. The present analysis expands these results by including the effect of different independent predictors in each gene, selected to be the strongest reported to date, in a homogeneous cohort of β^0-thalassemia patients. This, we believe, enables a better definition of the respective effects of each predictor. Above all this work relates genetic modifiers to time to first transfusion, a key event that characterizes disease severity regardless of patients' major or intermedia phenotype, thus notably increasing our knowledge on the specific effects of genetic modifiers of the clinical severity of β-thalassemia.

While it is likely that future whole genome sequencing studies will better define the genetic polymorphisms that modulate the effect of the *BCL11A* and *HBS1L-MYB* loci, the results from the present study could already be of support in clinical settings, by providing clear probabilities for the need to start transfusion at different ages as a function of the personal genetic background of individual patients.

Authorship and Disclosures

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

Financial and other disclosures provided by the authors using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are also available at www.haematologica.org.

References

1. Cao A, Galanello R. Beta-Thalassemia. In: Pagon RA, Bird TC, Dolan CR, Stephens K, editors. GeneReviews [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2000 [updated 2010].
2. Cao A, Rosatelli C, Pirastu M, Galanello R. Thalassemias in Sardinia: molecular pathology, phenotype-genotype correlation, and prevention. Am J Pediatr Hematol Oncol. 1991;13(2):179-88.
3. Taher A, Musallam KM, Cappellini MD. Thalassaemia intermedia: an update. Mediterr J Hematol Infect Dis. 2009;1(1): e2009004.
4. Cao A, Galanello R, Rosatelli MC. Genotype-phenotype correlations in beta-thalassemias. Blood Rev. 1994;8(1):1-12.
5. Lettre G, Sankaran VG, Bezerra MAC, Araújo AS, Uda M, Sanna S, et al. DNA polymorphisms at the BCL11A, HBS1L-MYB, and beta-globin loci associate with fetal hemoglobin levels and pain crises in sickle cell disease. Proc Natl Acad Sci USA. 2008; 105(33):11869-74.
6. Sedgewick AE, Timofeev N, Sebastiani P, So JCC, Ma ESK, Chan LC, et al. BCL11A is a major HbF quantitative trait locus in three different populations with [beta]-hemoglobinopathies. Blood Cells Mol Dis. 2008;41 (3):255-8.
7. Garner C, Tatu T, Reittie JE, Littlewood T, Darley J, Cervino S, et al. Genetic influences on F cells and other hematologic variables: a twin heritability study. Blood. 2000;95(1): 342-6.
8. Creary LE, Ulug P, Menzel S, McKenzie CA, Hanchard NA, Taylor V, et al. Genetic variation on chromosome 6 influences F cell levels in healthy individuals of African descent and HbF levels in sickle cell patients. PLoS One. 2009;4(1):e4218.
9. So CC, Song YQ, Tsang ST, Tang LF, Chan AY, Ma ES, et al. The HBS1L-MYB intergenic region on chromosome 6q23 is a quantitative trait locus controlling fetal haemoglobin level in carriers of beta-thalassaemia. J Med Genet. 2008;45(11):745-51.
10. Menzel S, Thein SL. Genetic architecture of hemoglobin F control. Curr Opin Hematol. 2009;16(3):179-86.
11. Galanello R, Sanna S, Perseu L, Sollaino MC, Satta S, Lai ME, et al. Amelioration of Sardinian beta0 thalassemia by genetic modifiers. Blood. 2009;114(18):3935-7.
12. Menzel S, Garner C, Gut I, Matsuda F, Yamaguchi M, Heath S, et al. A QTL influencing F cell production maps to a gene encoding a zinc-finger protein on chromosome 2p15. Nat. Genet. 2007;39(10):1197-9.
13. Solovieff N, Milton JN, Hartley SW, Sherva R, Sebastiani P, Dworkis DA, et al. Fetal hemoglobin in sickle cell anemia: genome-wide association studies suggest a regulatory region in the 5' olfactory receptor gene cluster. Blood. 2010;115(9):1815-22.
14. Bhatnagar P, Purvis S, Barron-Casella E, Debaun MR, Casella JF, Arking DE, et al. Genome-wide association study identifies genetic variants influencing F-cell levels in sickle-cell patients. J. Hum. Genet. 2011;56 (4):316-23.
15. Galarneau G, Palmer CD, Sankaran VG, Orkin SH, Hirschhorn JN, Lettre G. Fine-mapping at three loci known to affect fetal hemoglobin levels explains additional genetic variation. Nat Genet. 2010;42(12): 1049-51.
16. Farrell JJ, Sherva RM, Chen Z-Y, Luo H-Y, Chu BF, Ha SY, et al. A 3-bp deletion in the HBS1L-MYB intergenic region on chromosome 6q23 is associated with HbF expression. Blood. 2011;117(18):4935-45.
17. Uda M, Galanello R, Sanna S, Lettre G, Sankaran VG, Chen W, et al. Genome-wide association study shows BCL11A associated with persistent fetal hemoglobin and amelioration of the phenotype of beta-thalassemia. Proc Natl Acad Sci USA. 2008;105(5):1620-5.
18. Sutton M, Bouhassira EE, Nagel RL. Polymerase chain reaction amplification applied to the determination of beta-like globin gene cluster haplotypes. Am J Hematol. 1989;32(1):66-9.
19. Galanello R, Sollaino C, Paglietti E, Barella S, Perra C, Doneddu I, et al. Alpha-thalassemia carrier identification by DNA analysis in the screening for thalassemia. Am J Hematol. 1998;59(4):273-8.
20. T. I. H. Consortium, The International HapMap Project. Nature. 2003;426(6968): 789-96.
21. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet. 2006;38:904-9.
22. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007;81(3):559-75.
23. Weatherall DJ, Clegg JB. The Thalassemia Syndromes. 4th 2001 Blackwell Science Ltd.
24. Badens C, Joly P, Agouti I, Thuret I, Gonnet K, Fattoum S, et al. Variants in genetic modifiers of beta-thalassemia can help to predict the major or intermedia type of the disease. Haematologica. 2011;96(11):1712-4.