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STUDY OF MOLECULAR CORRELATION OF RS11886868 LOCUS IN BCL11A GENE POLYMORPHISMS WITH β-THALASSAEMIA

Alyaa Saad Abed[1]*, Taif Majid Abed Al hussein[2] and Zainb Mohammed Jassim[1]

[1*]Department of Genetic engineering, Biotechnologies College, University of Al-Qasim green, Iraq.
[2]Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Iraq.

Abstract

In Beta-thalassaemia, is the clinical phenotype that is highly variable, that is ranging from relatively mild to severe anaemia, which caused as a result of environmental and genetic factor. The insistence HbF ($\alpha 2\beta 2$) production represents one of the major causes of clinical severity of Beta-thalassaemia. HbF is the predominant haemoglobin generated during fetal life which replaced to adult haemoglobin (HbA, $\alpha 2\beta 2$) around birth.

By the end of the first year of life the levels of HbF declined to less than 1% of total haemoglobin. In some persons haemoglobin replacement is not complete and a significant quantities of HbF is produced during maturity, resulting in a condition called hereditary persistence of fetal haemoglobin (HPFH). However, HbF levels are also affected by genetic factors outside of the β-globin gene cluster. Genetic association studies have identified SNPs inmajor loci that are associated with the variation of HbF levels in patients with β-thalassaemia and in healthy adults. Some of these loci are the BCL11A gene onchromosome 2 (2p16.1).

A case control study involving 75 Beta-thalassaemia patient and 40 healthy was conducted. PCR-RFLP for BCL11A geneis used to estimate the molecular correlation with the influence and consequence of Beta-thalassaemia. This polymorphism are correlated with the advanced risk of acute leukemia. The present study indicates observed strong association of rs11886868 locus in BCL11A gene and effect on HbF and Beta-thalassaemia development. The genetic detaction for rs11886868 locus in BCL11A gene show the a strong significance between the gene polymorphism and Beta-thalassaemia in this gene site. This study revealed that there was significant association of rs11886868 locus/BCL11A gene, and disease incidence, and the results appear that b allele is responsible for disease development.

Introduction

Beta-thalassaemia is a common genetically inherited autosomal defect that is distributed among populations in all of the world. A uses of Beta -thalassaemia is illustrated due to mutations that lead to low or complete absence of expression of the β-globin gene (Pavlos *et al.,* 2014).

In Beta-thalassaemia large amount of unbound alpha globin chains precipitate in the red cell precursors which leading to their destruction in the bone marrow and so resulting in ineffective erythropoiesis. Also, large amount of unbound alpha globin chains leading to induced amage of membrane in mature thalassaemic red cells that resulting to haemolysis (Galanello, 2010).

***Author for correspondence:** E-mail: saadaliaa@yahoo.com

In Beta-thalassaemia, is the clinical phenotype that is highly variable, ranging from relatively mild to severe anaemia, due to environmental and genetic factors (Pavlos *et al.,* 2014 and Ingram, 1959).

The persistence production of HbF ($\alpha 2\beta 2$) represents one of the major ameliorating factors of clinical severity of Beta-thalassaemia (Stamatoyannopoulos, 2005). HbF is the predominant haemoglobin produced during fetal life which replaced to adult haemoglobin (HbA, $\alpha 2\beta 2$) around birth (Bauer *et al.,* 2012).

By the end of thefirst year of life the levels of HbF decrease to less than 1% of total haemoglobin. In some peoples haemoglobin replacement is not complete and a significant amount of HbF is produced during puberty,

resulting in a condition called hereditary persistence of fetal haemoglobin (HPFH) (Thein and Craig, 1998).

In β-thalassaemia, high γ-globin expression inhibits HbS polymerization and α-globin precipitation, respectively (Akinsheye, 2011).

The increased levels of HbF may be due to point mutations at the promoter of the γ-globin gene and deletions within the β-globin gene cluster (Pavlos *et al.,* 2014).

However, HbF levels are also affected by genetic factors outside of the β-globin gene cluster. Genetic association studies have identified SNPs inmajor loci that are associated with the variation of HbF levels in patients with β-thalassaemia and in healthy adults. These loci are the BCL11A gene onchromosome 2 (2p16.1) and the HBS1L-MYB intergenicregion on chromosome 6 (6q23.3) (Thein *et al.,* 2007; Menzel *et al.,* 2007).

The polymorphisms of BCL11A gene that have been described and are associated with variable HbF levels are located within the second intron of this gene. The HBS1L-MYB intergenicpolymorphisms (HMIP) are present in three linkage disequilibrium (LD) blocks with most of the effect on HbF levels and numbers of F cells contributed by the second block (Thein *et al.,* 2007; Menzel *et al.,* 2007; Galarneau *et al.,* 2010; Lettre *et al.,* 2008; So *et al.,* 2008).

The BCL11A gene encodes a zinc finger transcription factor that represses HbF synthesis (γ-globin expression) (Pavlos *et al.,* 2014; Xu *et al.,* 2010). Expression of the two major isoforms (XL, L) of BCL11A protein in human adult erythroid cells is needed to repress the γ-globin expression (Jawaid *et al.,* 2010; Dadheech *et al.,* 2016).

The BCL11A protein occupies sites within the locus control region (LCR) and intergenic regions of the β-globin locus but does not associate with the promoter of the γ-globin genes indicating that the regulation of γ-globin expression is a complex process (Jawaid *et al.,* 2010).

Thus, analysis of SNPs that are located in the BCL11 Agene and the HBS1L-MYB intergenic region and have an effect on HbF levels can provide crucial genetic information enabling patient stratification and can help predict the severity of disease in new borns with β-thalassaemia[1].

Materials and Methods

75 acute β-thalassaemia cases and 40 control samples were used in this study. Blood specimen was extracted from diagnostic patients that carry β-thalassaemia, which obtained from Babylon birth and children hospital. The age and sex control samples were included (1-6 years), male and female. Genomic DNA was isolated by extraction by special purification kit (Faverogen) due to the manufactures protocol.

Genotyping methods

Specimens of all patients and controls that carry rs11886868 locus in BCL11A genepolymorphism were identified by conventional. PCR-RFLP methods (Jamroziak *et al.,* 2004).

rs11886868 locus in BCL11A genepolymorphism, was. detected by PCR amplification by using the following primers: forward, primer 5' TTTGGTGCTACCCTG AAAGAC3' and reverse primer 5' ACTCAACAGTA GCAGAATGAAAGAG 3'Dadheech *et al.,* (2016). PCR, product was carried out in, 50μL reaction mixture containing PCR buffer (10mM Tris-HCl, pH 9.0 50mM KCl, 1.5mM MgCl2) 200 μM of each, dNTP, 1 unit of Taq DNA polymerase (due to, Biotech, USA), 20μM of each primer and 100ng of genomic DNA. PCR conditions was primary, denaturation at 94C. for 60s, annealing at 60C for, 60s, extension at 72C for 60s, and final extension 72C for 5min. PCR product of (540), bp digested with restriction enzymes *MboI*, incubated at 37C for 24hr homozygote (bb) 470 and 70bp fragments, polymorphic homozygote (BB) with 540bp fragments and heterozygote (Bb) with 540, 470, and 70bp fragments and then separated by agarose gel electrophoresis 2%, and staining

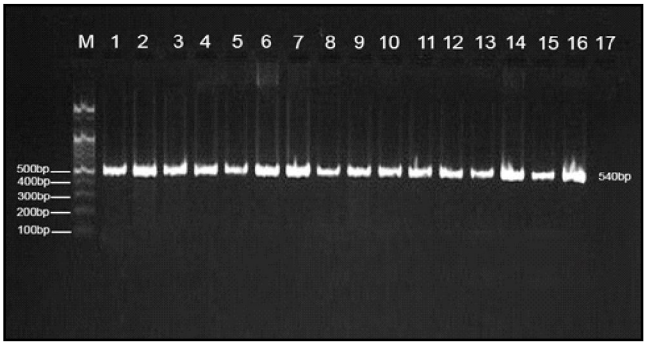

Fig. 1: electrophoresis pattern of PCR product of BCL11Agene (540bp), the optimum annealing temperature was 62.0ºC

by Ethidium bromide that used for visualized.

Results and Discussion

BCL11A genotyping PCR

The PCR product of BCL11 Agene amplification was 540bp Fig. 1.

The Genotype of BCL11Agene polymorphism using PCR-RFLP

The Genotype of rs11886868 locus/BCL11A

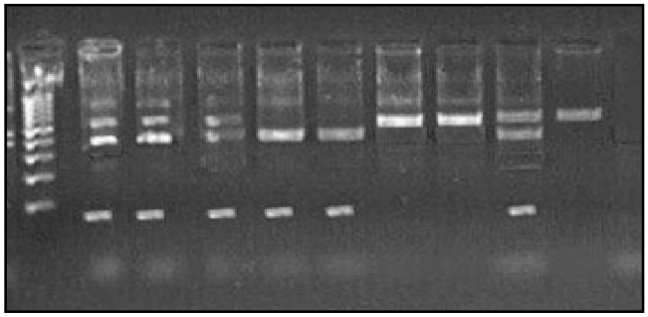

Fig. 2: Electrophoresis pattern of PCR-RFLP by 2% agarose gel for PCR product of rs11886868 locus in BCL11A gene (540 bp) with restriction enzyme *MboI*. Lane (1) DNA ladder. Lane (6, 7, 9): control. Lane (6, 7, 9) represented homozygote(BB) genotype(540bp). Lane(1-3, 8) Heterozygote (Bb) genotype(540, 470, 70 bp), Lane (4,5) homozygote (bb) genotype(470, 70). Lane: (1-5,8) patients.

genepolymorphism with Allele frequency between the two group control and patient group were detected using PCR-RFLP technique. Results from Fig. 2 show the genotype of BCL11Agene in the two study groups control and patients (the control were 40 samples while the patients were 75 samples), BB homogenotype represented (540bp), Bb The heterogenotype represented (540bp, 470bp and 70 bp), and bb the homogenotype represented (470bp and 70bp).

Table 1: Genotype of rs11886868 locus in BCL11A genepolymorphism with Allele frequency.

Genotype BCL11A	Control	Patient
BB	31(77.5%)	3(4%)
Bb	7(17.5%)	15(20%)
bb	2(5%)	57(76%)
Total number	40(100%)	75(100%)
Allele frequency		
Allele	**Control**	**Patient**
B	0.9	0.14
b	0.1	0.86

The genotype frequencies of BB, Bb, and bb of rs11886868 locus/BCL11A genepolymorphism were 3(4%), 15(20%) and 57(76%) in the patient group, while 31(77.5%), 7(17.5%) and 2(5%) in the control group, table 1 and Fig. 2.

The data of allele frequencies of point mutations on rs11886868 locus in BCL11A genein two study group control and patients are presented in table 1. For patient groups the allele frequency of (B) variant allele was 0.86, but (b) allele variant frequency was 0.14 according to Hardy- Wienberg equation While for control groups the allele frequency of (B) variant allele was 0.9 but (b) allele variant frequency was 0.1 according to Hardy-Wienberg equation.

Fig. 1 show the PCR product of BCL11A gene amplification was 540bp Dadheech *et al.,* (2016).

Results from table 1 and Fig. 2 show that there was significant association of rs11886868 locus/BCL11A gene, and disease incidence, and the results appear that b allele is responsible for disease development.

The result of this study was with agreement of that of Dadheech, *et al.* (2016).

The role of BCL11A as a regulator of γ-globin gene silencing has also been demonstrated experimentally by increased production of HbF in developing adult erythroblasts after small hairpin RNA (sh-RNA) mediated knocked down19. BCL11A mediated silencing is orchestrated through cooperation with a high mobility group transcription factor SOX6, since BCL11A and SOX6 are co-expressed and interact physically 16, 19. The data from genetic and functional studies support a key role for BCL11A in silencing of γ-globin genes during the developmental switching as well as its potential role in reactivation of HbF in adult erythroblasts Dadheech *et al.,* (2016).

References

Akinsheye, I., A. Alsultan, N. Solovieff, D. Ngo, C.T. Baldwin, P. Sebastiani, D.H. Chui, M.H. Steinberg: Fetal hemoglobin in sickle cell anemia. *Blood 2011,* **118(1):** 19–27.

Bauer, D.E., S.C. Kamran, S.H. Orkin: Reawakening fetal hemoglobin: prospects for new therapies for the beta-globin disorders. *Blood 2012,* **120(15):** 2945–2953.

Galarneau, G., C.D. Palmer, V.G. Sankaran, S.H. Orkin, J.N. Hirschhorn, G. Lettre: Fine-mapping at three loci known to affect fetal hemoglobin levelsexplains additional genetic variation. *Nat. Gen. 2010,* **42(12):** 1049–1051.

Galanello, R., Origa R: Beta-thalassemia. *Orphanet J. Rare Dis. 2010,* **5:** 11.

Ingram, V.M: Separation of the peptide chains of human globin. *Nature 1959,* **183:** 1795–1798.

Jawaid, K., K. Wahlberg, S.L. Thein, S. Best: Binding patterns of BCL11A in the globin and GATA1 loci and characterization of the BCL11A fetalhemoglobin locus. *Blood Cells Mol. Dis. 2010,* **45(2):** 140–146.

Lettre, G., V.G. Sankaran, M.A. Bezerra, A.S. Araujo, M. Uda, S. Sanna, A. Cao, D. Schlessinger, F.F. Costa, J.N. Hirschhorn, *et al.,*: DNA polymorphisms at the BCL11A, HBS1L-MYB, and beta-globin loci associate with fetalhemoglobin levels and pain crises in sickle cell disease. *Proc. Natl. Acad. Sci.* USA 2008, **105(33):** 11869–11874.

Menzel, S., C. Garner, I. Gut, F. Matsuda, M. Yamaguchi, S. Heath, M. Foglio, D. Zelenika, A. Boland, H. Rooks, *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):** 1197–1199.

Pavlos Fanis, Ioanna Kousiappa, Marios Phylactides and Marina Kleanthous (2014). Genotyping of BCL11A and HBS1L-MYB SNP associated with fetal haemoglobin levels: a SNaP shot mini sequencing approach. Fanis *et al., BMC Genomics 2014,* **15:** 108.

Sneha Dadheech, A., B.D. Madhulatha, A. Suman Jain, C. James Joseph, A.A. Jyothy and Anjana Munshi (2016). Association of BCL11A genetic variant (rs11886868) with severity in β-thalassaemia major & sickle cell anaemia. *Indian J. Med. Res. 2016 Apr.,* **143(4):** 449–454.

So, C.C., Y.Q. Song, S.T. Tsang, L.F. Tang, A.Y. Chan, E.S. Ma, L.C. Chan: The HBS1LMYB intergenic region on chromosome 6q23 is a quantitative trait locus controlling fetal haemoglobin level in carriers of beta-thalassaemia. *J. Med. Gen. 2008,* **45(11):** 745–751.

Stamatoyannopoulos, G: Control of globin gene expression during development and erythroid differentiation. *Exp. Hematol 2005,* **33(3):** 259–271.

Thein, S.L., J.E. Craig: Genetics of Hb F/F cell variance in adults and heterocellular hereditary persistence of fetal hemoglobin. *Hemoglobin 1998,* **22(5–6):** 401–414.

Thein, S.L., S. Menzel, X. Peng, S. Best, J. Jiang, J. Close, N. Silver, A. Gerovasilli, C. Ping, M. Yamaguchi, *et al.,*: Intergenic variants of HBS1L-MYB are responsible for a major quantitative trait locus on chromosome 6q23 influencing fetalhemoglobin levels in adults. *Proc. Natl. Acad. Sci.* USA 2007, **104(27):** 11346–11351.

Xu, J., V.G. Sankaran, M. Ni, T.F. Menne, R.V. Puram, W. Kim, S.H. Orkin: Transcriptional silencing of {gamma}-globin by BCL11A involves long-rangeinteractions and cooperation with SOX6. *Genes. Dev. 2010,* **24(8):** 783–798.