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

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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 (α2β2) 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, α2β2) 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 in major 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 on chromosome 2 (2p16.1).

A case control study involving 75 Beta-thalassaemia patient and 40 healthy was conducted. PCR-RFLP for BCL11A gene is 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 detection 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).

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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 in major 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 on chromosome 2 (2p16.1) and the HBS1L-MYB intergenic region 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 intergenic polymorphisms (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 BCL11A gene 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 gene polymorphism were identified by conventional. PCR-RFLP methods (Jamroziak et al., 2004).

rs11886868 locus in BCL11A gene polymorphism, was detected by PCR amplification by using the following primers: forward, primer 5’ TTTGGGTGCTACCTCTG AAAGAC3’ and reverse primer 5’ ACTCAACAGTAGCAGATGAAAGAG 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 94°C for 60s, annealing at 60°C for 60s, extension at 72°C for 60s, and final extension 72°C for 5min. PCR product of (540), bp digested with restriction enzymes MboI, incubated at 37°C 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 by Ethidium bromide that used for visualized.

**Results and Discussion**

**BCL11A genotyping PCR**

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

**The Genotype of BCL11A gene polymorphism using PCR-RFLP**

The Genotype of rs11886868 locus/BCL11A...
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 \( \gamma \)-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 \( \gamma \)-globin genes during the developmental switching as well as its potential role in reactivation of HbF in adult erythroblasts Dadheech et al., (2016).

References


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


