DNA Methylation of *IGSF4* gene as an Epigenetic Modifier in HbE/β-Thalassaemia

Haiyuni Mohd Yassim[1], Wan Zaidah Abdullah[1], Muhammad Farid Johan[1*]

[1]Department of Haematology, School of Medical Sciences, Universiti Sains Malaysia,16150 Kubang Kerian, Kelantan, Malaysia.

The large clinical spectrum of Haemoglobin E (HbE)/β-thalassaemia leads to identification of modifiers that cause the complexity[1]. *IGSF4*, a member of the immunoglobulin superfamily 4 is known as a thalassaemia-related gene that plays an important role in globin synthesis. Methylation of *IGSF4* was reported to interrupt the process of globin synthesis through its interaction with other genes in the regulation network of globin expression[2]. Specific cells isolation is needed in order to study the methylation profile as the interaction between various haematopoietic cells including nucleated red blood cells (NRBCs) in whole blood could impact the methylation results[3]. Therefore, the objective of this study was to describe the pattern of DNA methylation at the promoter region of *IGSF4* gene that may involve in the alteration of globin synthesis in HbE/β-thalassaemia patients.

Nucleated red blood cells (NRBCs) were isolated from 6mL peripheral blood of thalassaemia patients after the isolation of mononuclear cells (MNCs) based on density gradient. Magnetic activated cell sorting (MACs) with anti-CD71 was used to enrich isolated NRBC and validated with flow cytometry (Becton-Dickson, USA). DNA was extracted from the samples and subjected for bisulfite modification using EZ DNA methylation-gold Kit. Methylation specific polymerase chain reaction (MS-PCR) and DNA sequencing were employed to screen and detect the methylation status targeting 10 CpG sites within the promoter region of *IGSF4* gene in HbE/β-thalassaemia patients with CD26/IVS1_1. One HbE/β-thalassaemia patient with CD26/IVS1_1MS-PCR showed visible bands in both methylated and unmethylated primer sets for *IGSF4* gene, indicating that the M1 region (-696 to -582 relative to the ATG) of *IGSF4* promoter is partially methylated (Fig. 1).

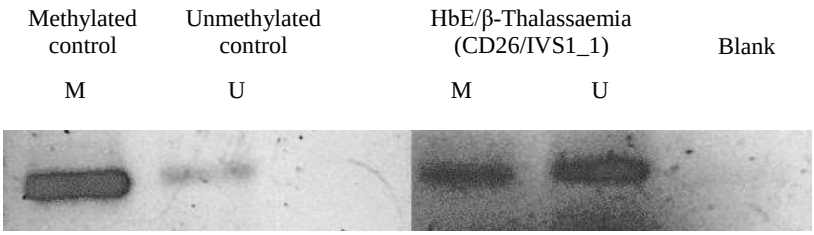

Fig. 1: Methylation-specific PCR amplification results of *IGSF4* gene at M1 region in HbE/β-thalassaemia patient. Universal methylated and non-methylated DNA were used as positive control for methylated and non-methylated primers. M, methylated; U, unmethylated.

Interestingly, in DNA sequencing, 5' cytosine of guanine (CpG) remained as such on bisulfite-modified DNA of HbE/β-thalassaemia denoted all CpGs in the amplified regions are methylated (Figure 2). This is parallel with previous findings which reported that the promoter of IGSF4 was highly methylated in thalassemia patients[2]. DNA methyltransferases (DNMTs), the key enzyme of genome methylation was engaged in regulating gene expression and repression epigenetically[4]. Thus, methylation of *IGSF4* promoter could repress the gene expression and interrupts the synthesis of β-globin chain.

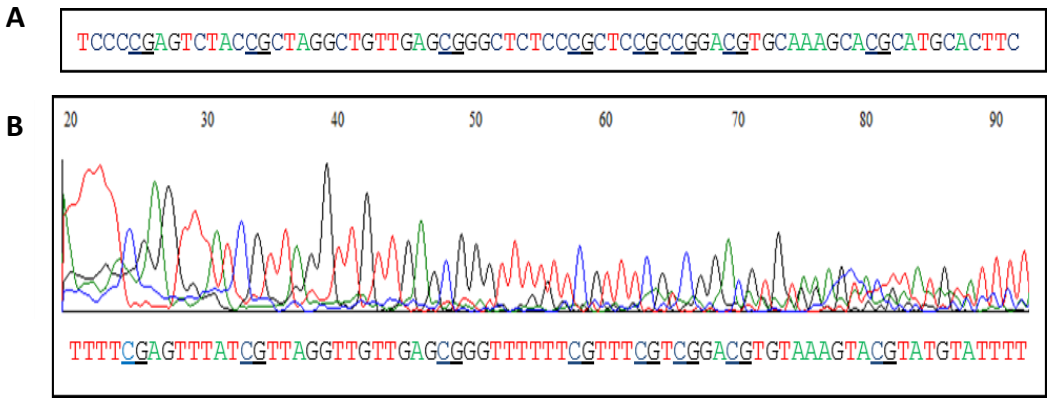

Fig. 2: DNA sequencing of IGSF4 gene. A. Unmodified or wild-type DNA sequenceof IGSF4 M1 region. B. Modified DNA of HbE/β-thalassaemia patient showed positively methylated with C remains as C in CpG dinucleotides. Positions of CpG sites were underlined with boldface type.

The mechanism of abnormal β-globin chain production could be from *IGSF4* promoter methylation. This probably contributes to one of the potential modifiers for HbE/β-thalassaemia from epigenetic perspective. Further investigation is required to confirm this finding.

Keywords: HbE/β thalassaemia, DNA methylation, *IGSF4*

*** Correspondence:** faridjohan@usm.my

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