



**UNIVERSITI PUTRA MALAYSIA**

**DETECTION OF  $\beta$ -GLOBIN GENE POLYMORPHISMS USING REAL TIME  
PCR-HIGH RESOLUTION MELTING METHOD IN SELECTED IRANIAN  $\beta$ -  
THALASSAEMIA PATIENTS**

**SEYED JALAL MARASHI**

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**By**

**SEYED JALAL MARASHI**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in  
Fulfilment of the Requirements for the Degree of Master of Science**

**April 2011**

## DEDICATIONS

**I would like to dedicate this thesis to:**

**My dear parents, who sacrificed their good life because of my progress and giving the hope and energy during working on this thesis.**

**"We all know that light travels faster than sound. That's why certain people appear bright until you hear them speak." — Albert Einstein**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

**DETECTION OF  $\beta$ -GLOBIN GENE POLYMORPHISM USING REAL TIME PCR-HIGH RESOLUTION MELTING METHOD IN SELECTED IRANIAN  $\beta$ -THALASSAEMIA PATIENTS**

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**April 2011**

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$\beta$ -thalassemia is a common autosomal recessive disorder among the hereditary diseases worldwide. It is caused by the reduced production of functional  $\beta$ -globin which lead to anemia, as a result of point mutations, small deletions or insertions within the  $\beta$ -globin gene which is located as a cluster on the short arm of chromosome 11. More than 200 different mutations of  $\beta$ -globin genes have been identified.  $\beta$ -thalassemia is most prevalent around the Mediterranean. The gene frequency of  $\beta$ -thalassemia in Iran is high and alters significantly from area to area, but around the Caspian Sea and Persian Gulf with more than 10% have the highest rate. Since the Iranian populations are mixture of different ethnic groups and regarding to lack of precise prevalence of common mutations in  $\beta$ -thalassemic patients in Qazvin province of Iran, research project was defined to identify an accurate allele frequency of common mutations in  $\beta$ -thalassemia patients, with Real Time-PCR HRM method.

PCR-based strategies and direct sequencing have been carried out to screen  $\beta$ -thalassemia subjects. In this research the Rotor-Gene™ 6000 real time rotary analyzer was applied to amplify a target sequence of DNA to high copy number with incorporation of fluorescent (EvaGreen™) dye prior to performing a High Resolution Melting (HRM) analysis. Samples were then analyzed in the HRM channel according to their dissociation behavior. In this descriptive-analytical study,  $\beta$ -thalassemia chromosomes of 120 affected patients (120  $\beta$ -thalassemia major) were evaluated. The most common mutation detected among subjects was nucleotide 1 (G to A conversion) of Intervening Sequence (IVS) region 2. Thus, IVS-II-I (G-A) (25.4%), is followed, based on frequency, with IVS-I-110(G-A) (15.4%), IVS-I-5(G-C) (13.3%), FSC-8/9(5.8%), FSC-36/37 (4.6%), Codon 30 (2.5%), IVS-I-6(T-C) (2.1%), IVS-I-1(G-A) (0.8%). The three mutations IVS-II-1(G-A), IVS-I-110(G-A) and IVS-I-5(G-C) accounted for about 54.2% of all of the mutations. The most common allele being IVS-II-I (G-A) with a frequency of 25.4%. In the rest of samples (29.2%) these 8 mutations were not detected and were remained unknown after analysis with common primer that needs further investigation which is beyond the objectives of the study. A rare Hb Monroe and codon 8 (-AA) mutations from Qazvin province of Iran were also detected. The results derived from HRM analysis were fully in accordance with sequencing. Real time- PCR was produced enough DNA for fluorescent melting analysis, both amplification and analysis could be performed in the same tube, providing a homogeneous, closed-tube system that requires no processing or separation steps without any contamination. Consequently HRM could be a sensitive, simpler and more cost effective way to characterize samples than conventional methods and HRM method could greatly facilitate screening for these 8  $\beta$ -thalassemia mutations. But the main limitation of HRM is that the precise mutation cannot be readily identified and it

thus needs to be coupled with sequencing method. We suggest this rapid and accurate method for molecular screening to detect the common  $\beta$ -thalassemia mutations in the Iranian population as well as in other ethnic groups and nationalities in which  $\beta$ -thalassemia alleles are prevalent.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**POLIMORFISMA GEN  $\beta$ -GLOBIN TERHADAP PESAKIT  $\beta$ -THALASSEMIA  
DARI SEBAHAGIAN WARGANEGARA IRAN DENGAN MENGGUNAKAN  
“REAL TIME PCR-HIGH RESOLUTION MELTING”**

Oleh

**SEYED JALAL MARASHI**

**April 2011**

**Pengerusi: Prof. Patimah Bt Ismail, PhD**

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$\beta$ -thalassemia merupakan sejenis penyakit gangguan autosom resesif yang biasa ditemui dalam penyakit keturunan di seluruh dunia. Gangguan ini disebabkan oleh kurang penghasilan gen  $\beta$ -globin yang seterusnya akan menyebabkan penyakit anemia. Penghasilan gen  $\beta$ -globin yang kurang adalah kesan daripada proses mutasi, pemotongan kecil atau penyisipan dalam gen  $\beta$ -globin yang terletak membentuk kluster pada lengan pendek kromosom 11. Lebih daripada 200 jenis mutasi gen  $\beta$ -globin yang berlainan telah ditemui.  $\beta$ -thalassemia merupakan gangguan gen yang paling tinggi berlaku di kawasan Mediteranian. Frekuensi gen  $\beta$ -thalassemia di kalangan penduduk Iran adalah tinggi dan berubah secara signifikan dari tempat ke tempat, namun di kawasan Laut Kaspia dan Teluk Parsi lebih daripada 10% mempunyai kadar  $\beta$ -thalassemia yang tertinggi. Memandangkan populasi di Iran adalah merangkumi pelbagai kumpulan etnik dan kadar mutasi dalam pesakit  $\beta$ -thalassemia di Qazvin, Iran

yang kurang tepat, maka kajian ini dijalankan untuk menentukan dengan tepat frekuensi alel bagi mutasi dalam pesakit  $\beta$ -thalassemia, menggunakan kaedah “Real Time PCR-High Resolution Melting” (Real Time-PCR HRM). PCR-berdasarkan strategi dan kaedah rangkaian secara langsung telah dijalankan untuk mengenalpasti subjek bagi  $\beta$ -thalassemia. Dalam kajian ini, penganalisis “Rotor-Gene™ 6000 real time rotary” telah digunakan untuk menguatkan jujukan DNA sasaran kepada nombor salinan yang lebih tinggi dengan penggunaan pewarna pendafluoran (EvaGreen™) sebelum diteruskan dengan analisis Pencairan Resolusi Tinggi (HRM). Kemudian, sampel telah dianalisis dalam saluran HRM berdasarkan sifat pengasingannya. Dalam kajian penghuraian-analitikal ini, kromosom  $\beta$ -thalassemia dari 120 orang pesakit yang terjejas (120  $\beta$ -thalassemia major) telah dianalisa. Mutasi yang paling banyak ditemui di kalangan subjek ialah nukleotida 1 (penukaran G kepada A) dari Rangkaian Pencelahan (IVS) bahagian 2 yang mana berdasarkan frekuensi, IVS-II-I (G-A) (25.4%), diikuti dengan IVS-I-110(G-A) (15.4%), IVS-I-5(G-C) (13.3%), FSC-8/9(5.8%), FSC-36/37 (4.6%), Codon 30 ( 2.5%), IVS-I-6(T-C) (2.1%) dan IVS-I-1(G-A) (0.8%). Tiga jenis mutasi iaitu IVS-II-1(G-A), IVS-I-110(G-A) dan IVS-I-5(G-C) merangkumi 54.2% dari kesemua mutasi. Dalam lebihan sampel (29.2%), 8 mutasi yang telah dikenalpasti tidak ditemui dan tetap tidak diketahui selepas analisa dijalankan menggunakan primer umum yang mana ianya memerlukan kajian yang lebih lanjut. Mutasi hemoglobin Monroe dan codon 8 (-AA) yang jarang berlaku di wilayah Qazvin juga telah ditemui. Keputusan-keputusan dari analisis HRM adalah sangat bertepatan dengan jujukannya. “Real time-PCR” telah menghasilkan DNA yang cukup bagi analisis pencairan pendafluor yang merangkumi amplifikasi dan analisis dalam tiub yang sama, sample yang homogenus, sistem tiub tertutup yang tidak memerlukan langkah-langkah pemprosesan atau pemisahan tanpa sebarang pencemaran. Oleh itu, HRM merupakan



salah satu kaedah yang lebih sensitif, mudah dan kurang kos bagi mengklasifikasikan sampel berbanding kaedah lama. Kaedah HRM juga dapat memudahkan penyaringan bagi 8 jenis mutasi  $\beta$ -thalassemia. Walau bagaimanapun, kelemahan utama HRM ialah ianya tidak dapat menentukan mutasi yang tepat dan ianya memerlukan kaedah rangkaian secara terus untuk mengatasi masalah ini. Kami mencadangkan agar kaedah yang cepat dan tepat bagi penyaringan molecular ini diaplikasikan dengan meluas bagi menentukan jenis mutasi yang biasa terjadi dalam populasi Iran dan kumpulan etnik yang lain serta negara-negara yang mempunyai kadar alel  $\beta$ -thalassemia yang tinggi.

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I certify that an Examination Committee has met on **19 April 2011** to conduct the final examination of **Seyed Jalal Marashi** on his Master thesis entitled **β-Globin Gene Polymorphisms in selected Iranian β-Thalassemia Patients Using Real Time PCR-High Resolution Melting** " in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the degree of Master.

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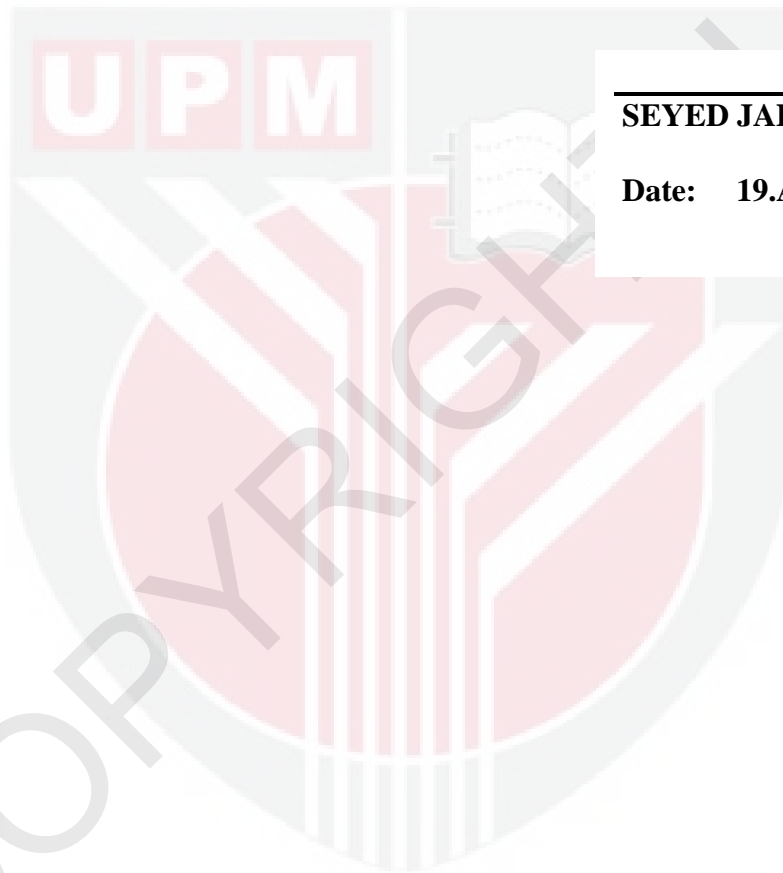
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## DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously and it is not any other institution concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



**SEYED JALAL MARASHI**

**Date: 19.Apr.2011**

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