UNIVERSITI PUTRA MALAYSIA

REAL-TIME HIGH RESOLUTION MELTING ANALYSIS-PCR FOR ASSESSMENT OF GENE MUTATIONS IN HUMAN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

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By

RUSNI BINTI MOHD JAS

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

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REAL-TIME HIGH RESOLUTION MELTING ANALYSIS-PCR FOR ASSESSMENT OF GENE MUTATIONS IN HUMAN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

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September 2011

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Faculty: Medicine and Health Sciences

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common hereditary renal diseases that affect 1 in 400 to 1 in 1000 life birth and cause by mutations in two known genes; *PKD1* and *PKD2*. Although the manifestation of Type1 ADPKD is similar to Type2, it is more severe and patients suffer total renal failure by the fifth decade of life. In finding the mutations occurring in both genes, PCR-based methodology have been used and all require technical expertise, otherwise are time consuming and costly. Therefore, this study aims to develop a much rapid screening

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procedure that is cost effective but have the same sensitivity to widely used methodology to differentiate ADPKD samples from control. Fourteen primers were selected and used to amplify the target genes by using Real-time High Resolution Melting Analysis PCR (Real-time HRMA PCR) and was compared to PCR-SSCP in terms of rapidity, sensitivity and reliability. PCR-SSCP revealed same number of bands in control and case sample, with different only in the bands' size. All primers successfully amplified respective genes in Real-time HRMA PCR. Ten primers had two apparent melting curves which enabled control and case samples to be differentiated. The mean melting temperature for control samples of the ten primers was 81.38±1.70^{o}C whereas in case samples the mean was 81.00±1.68^{o}C. The *p* value was 0.001. The melting curves of control were all shifted to the right having higher melting temperature while case melting curves were shifted to the left due to lower melting temperature. Possible mutations were found to be present in five of these primers due to lone case samples where the melting curve were shifted differently from control and case melting curves and cannot be classified either in control or case group ($p<0.001$). Three primers were found to be less useful to be used in Real-time HRMA PCR as control and case samples melted in the same manner with same exact temperature, resulting in inconclusive results. While other primers conferred single melting domain, a primer amplifying the *PKD1* gene was melted twice and evidence in both samples. The PCR-SSCP needs more time to be completed (mean; 13.93±0.94 hours) before analysis can be done while the Real-time HRMA PCR can be completed in a very short time (mean; 2.40±0.54 hours) including the analysis. Both methodologies are able to discriminate case from control but Real-time HRMA PCR is more rapid ($p<0.001$). Since Real-time

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HRMA PCR is more rapid and less procedure is needed, it is proven to be better for gene mutation screening. In conclusion, the Real-time HRMA PCR is not only sensitive, but it can screen for control and ADPKD samples more rapidly and more reliable with the application of commonly used primers in conventional PCR.

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

REAL-TIME HIGH RESOLUTION PCR UNTUK PENYARINGAN MUTASI GEN PESAKIT AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

Oleh

RUSNI BINTI MOHD JAS

September 2011

Pengerusi: Patimah Ismail, PhD

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Autosomal dominant polycystic kidney disease (ADPKD) merupakan salah satu penyakit genetik buah pinggang yang paling biasa dialami dengan nisbah 1 dalam 400 atau 1 dalam 1000 kelahiran hidup dan penyakit ini disebabkan mutasi yang berlaku kepada dua gen yang telah dikenalpasti; gen *PKD1* dan gen *PKD2*. Walaupun ciri-ciri Jenis1 dan Jenis2 adalah serupa, Jenis1 lebih serius dan pesakit akan mengalami kegagalan buah pinggang yang memerlukan rawatan dialisis sepanjang hayat semasa

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mereka berumur lewat 50-an. Untuk mengenalpasti mutasi yang berlaku di dalam kedua-dua gen tersebut, teknologi yang berasaskan PCR telah digunakan dan kesemua teknologi ini memerlukan kemahiran teknikal, paling tidak pun memerlukan banyak masa dengan kos yang mahal. Oleh itu, tujuan utama kajian ini adalah untuk membina-uji satu kaedah yang lebih pantas dan efektif bukan sahaja daripada segi kos, tetapi juga mempunyai sensitiviti yang setaraf dengan kaedah yang biasa digunakan untuk membezakan sampel pesakit ADPKD daripada sampel kawalan. Sebanyak 14 primer dipilih dan digunakan untuk kaedah Real-time HRMA PCR dan dibandingkan dengan kaedah PCR-SSCP daripada segi kepantasan, sensitiviti dan '*realibility*'. Hasil PCR-SSCP menunjukkan kehadiran jalur-jalur DNA dalam jumlah yang sama diantara pesakit dan kawalan, cuma berbeza daripada segi saiz jalur. Kesemua primer berjaya digunakan di dalam kaedah Real-time HRMA PCR. Sepuluh primer mempunyai dua lengkung pencairan yang berbeza, setiap satu mewakili sampel pesakit dan kawalan yang membolehkan perbezaan sampel dilakukan. Purata suhu pencairan bagi kawalan adalah 81.37±1.70^{o}C manakala bagi sampel pesakit adalah 81.01±1.68^{o}C. Lengkung pencairan untuk kawalan beranjak lebih ke kanan kerana suhu pencairan yang lebih tinggi manakala lengkung pencairan pesakit beranjak ke kiri disebabkan suhu pencairan yang lebih rendah (p<0.001). Tiga primer didapati tidak boleh digunakan dalam kaedah Real-time HRMA PCR kerana sampel pesakit dan kawalan mempunyai suhu dan lengkung pencairan yang sama menyebabkan tiada kesimpulan dapat dibuat. Hanya satu primer didapati mempunyai dua domain pencairan; kedua-dua sampel pesakit dan kawalan dicairkan dua kali, sementara yang lain hanya mempunyai satu domain pencairan. PCR-SSCP memerlukan lebih masa untuk disiapkan (purata masa;

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13.93±0.94 jam) sementara Real-time HRMA PCR memerlukan masa yang sangat singkat (purata masa; 2.40±0.54 jam). Kedua-dua cara berupaya untuk membezakan sampel pesakit daripada sampel kawalan tetapi Real-time HRMA PCR adalah lebih pantas (p<0.001). Disebabkan kepantasan dan kurangnya prosedur eksperimen, Real-time HRMA PCR dibuktikan lebih baik untuk penyaringan mutasi gen. Sebagai kesimpulan, Real-time HRMA PCR bukan sahaja sensitif, tetapi ia boleh menyaring sampel kawalan dan pesakit ADPKD dengan lebih cepat dan *'reliable'* menggunakan primer sedia ada yang digunakan di dalam PCR biasa.

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I certify that a Thesis Examination Committee has met on 30 September 2011 to conduct a final examination of Rusni binti Mohd Jas on her thesis entitled "**Real-time High Resolution Melting Analysis-PCR for Assessment of Gene Mutations in Human Autosomal Dominant Polycystic Kidney Disease**" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.A(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of **Master of Science**. The members of the Supervisory Committee were as follows:

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DECLARATION

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

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