ABSTRAK

Gen *Protein Kinase AMP-Activated Catalytic Subunit Alpha2 (PRKAA2)* merupakan gen yang mengkode enzim AMPKα2. Pada gen *PRKAA2* terdapat SNPs rs706466 yang terjadi di posisi 3'UTR dimana adenin (A) mengalami perubahan menjadi guanin (G) atau adenin (A) menjadi tiamin (T) dengan frekuensi yang cukup tinggi pada populasi Asia yaitu 47,6%. Penelitian terhadap SNPs gen *PRKAA2* rs706466 belum pernah dilakukan di Indonesia. Identifikasi SNPs dilakukan dengan metode PCR-RFLP. Pengembangan metode melalui optimasi diperlukan agar memberikan hasil optimum dan proses deteksi dapat berjalan cepat dan tepat. Kondisi optimum perlu divalidasi agar menghasilkan produk PCR yang spesifik dan reprodusibel.

Jenis penelitian ini yaitu deskriptif observasional dengan rancangan penelitian *case report*. Isolat DNA diambil dari populasi subjek uji pasien riwayat DM tipe 2 di salah satu Rumah Sakit swasta Yogyakarta. Isolat DNA dianalisis kualitatif menggunakan metode elektroforesis dengan media gel *agarose* 1%. Primer *forward*, primer *reverse* dan reagen yang digunakan berturut-turut yaitu '5-CCATAGGTTGATACATTGACCCA-3', 5'-TGGTGTGCAGAATACCAGCA-3', Promega *Go Taq Green Master Mix*. Optimasi suhu *annealing* dilakukan pada variasi suhu 55;56,1;57,9;60,7;64;67;68,9;70℃, sedangkan kadar primer pada variasi 2; 2,5; 3; 3,5 µM. Hasil optimasi dianalisis menggunakan elektroforesis dengan media gel *agarose* 1,5%. Hasil penelitian menunjukkan bahwa kondisi optimum PCR meliputi suhu *annealing* dan kadar primer, yaitu suhu *annealing* 56,1ºC dan kadar primer 3 µM. Kondisi optimum ini dapat menghasilkan produk PCR yang memenuhi parameter spesifisitas dan reprodusibilitas pada validasi metode sehingga dapat digunakan untuk identifikasi SNPs gen *PRKAA2* rs706466 dengan metode PCR-RFLP. Hasil identifikasi menunjukkan bahwa ketujuh sampel memiliki SNPs genotipe AG (heterozigot) yang menyebabkan adenin berubah menjadi guanin.

Kata Kunci: *PRKAA2*, SNPs, PCR, Optimasi, Validasi

ABSTRACT

The protein kinase gene AMP-Activated Catalytic Subunit Alpha2 (PRKAA2) is a gene that encodes the AMPKα2 enzyme. In the PRKAA2 gene, there are SNPs rs706466 that occur at the 3'UTR position where adenine (A) changes to guanine (G) or adenine (A) to thiamine (T) with a fairly high frequency in Asian populations, namely 47.6%. Research on the SNPs of the PRKAA2 rs706466 gene has never been conducted in Indonesia. Identification of SNPs was carried out by the PCR-RFLP method. The development of methods through optimization is needed in order to provide optimum results and the detection process can run quickly and precisely. Optimum conditions need to be validated in order to produce specific and reprodusibel *PCR products.*

This type of research is descriptive observational with a case report research design. DNA isolates were taken from the test subject population of patients with a history of type 2 diabetes at a private hospital in Yogyakarta. DNA isolates were analyzed qualitatively using electrophoresis method with 1% agarose gel as a medium. The forward primer, reverse primer and reagents used are '5-CCATAGGTTGATACATTGACCCA-3', 5'-TGGTGTGCAGAATACCAGCA-3', Promega Go Taq Green Master Mix. The annealing temperature optimization was carried out at a temperature variation of 55; 56.1; 57, 9; 60.7; 64; 67; 68, 9; 70℃, while the primary content was in variation 2; 2.5; 3; 3.5 M. The optimization results were analyzed using electrophoresis with 1.5% agarose gel as a medium. The results showed that the optimum conditions for PCR included annealing temperature and primer content, namely annealing temperature of 56.1oC and primer content of 3 M. This optimum condition can produce a PCR product that meets the parameters of specificity and reproducibility in the validation method so that it can be used to identify SNPs of the PRKAA2 rs706466 gene by the PCR-RFLP method. The identification results showed that the seven samples had SNPs genotype AG (heterozygous) which caused adenine to turn into guanine.

Keywords: *PRKAA2, SNPs, PCR, Optimization, Validation*