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A Novel Single Nucleotide Polymorphism, IVS2 -97A>T, in the Prostaglandin F2α Receptor Gene Was Identified among the Malaysian Patients with Glaucoma

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The Prostaglandin F2α (PGF2α) receptor gene has been found to play an important role in reducing the intraocular pressure of the glaucomatous patients. Variations of the PGF2α receptor gene may be responsible for the differences in the response to an antiglaucoma drug, Latanoprost. A combined method of denaturing High Performance Liquid Chromatography (dHPLC) and sequencing was applied to detection of the PGF2α receptor gene variant among the 76 Malaysian patients with glaucoma, and a novel single nucleotide polymorphism (SNP), IVS -97A>T, was identified. According to the genotyping analysis, 36.8% of the subjects were heterozygous for the variant allele T, while 9.2% homozygous. The frequency of variant allele T was 0.28. Although with a limited number of samples, our data suggested that this polymorphism is common in the Malaysian patients with glaucoma.

INTRODUCTION

The Prostaglandin F2α (PGF2α) receptor gene, located at chromosome 1p31.1, is approximately 40 kb in size and contains 3 exons (NCBI GenBank, AL136324). It has been found to play an important role in reducing the intraocular pressure through increasing uveoscleral outflow of aqueous humor in glaucomatous patients (1,5). Recently, the analogue of PGF2α, Latanoprost, has gained popularity and proven to be effective in the pressure lowering effect in many glaucoma patients (8). However, poor responsiveness has been observed in some of the patients (4,8).

In view of the exponential increase in the number of variants, including single nucleotide polymorphisms (SNPs) which may affect the responsiveness to treatment, there arises a need for the development of high throughput methods for polymorphisms detection. Among the methods available to date, denaturing high performance liquid chromatography (dHPLC) has emerged as a sensitive method (12).

In this study, we applied the dHPLC method for variant screening of the PGF2α receptor gene, and identified a novel SNP, IVS2 -97A>T, among the glaucomatous patients attending the Ophtalmology Clinic at Hospital Universiti Sains Malaysia (HUSM).

MATERIALS AND METHODS

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Study subjects. Seventy-six glaucomatous patients were recruited from the Ophthalmology Clinic, HUSM. Genomic DNA was extracted from venous blood using the QiaAmp MiniKit (Qiagen, Hilden, Germany). This study was approved by the Research and Ethics Committee of Universiti Sains Malaysia (USM), Health Campus, Malaysia. Informed written consent was obtained from all subjects.

PCR amplification. The reference sequence (i.e. wild type sequence) of PGF2α receptor gene was extracted from the NCBI GenBank (AL136324, http://www.ncbi.nlm.nih.gov/). Specific primer pair (F: tcatttgatttctttctgtcagtat; R: ccacacagattttactgtcctatta) were designed to amplify first the exon 3 of the gene using Primer3 software (10). DNA was subjected to 40 cycles of PCR amplification in a 20 µl total reaction volume containing 1.8 mM $MgCl_2$, 1 X Reaction Buffer (100 mM Tris-HCl, pH 8.3; 500 mM KCl), 0.375 mM dNTPs, 7.5 ρmol of each forward and reverse specific primers, ~100 ng template DNA and 1 unit of DNA polymerase. The PCR conditions are as follows: 96°C of pre-denaturation (5 min), 95°C of denaturation (60 s), 50°C of annealing (60 s) and 72°C of extension (90 s) followed by 72°C of final extension (5 min) in a thermal cycler PTC200 (MJ Research, Watertown, Massachusetts). The presence of amplicon was confirmed by gel electrophoresis on 2% agarose gel.

dHPLC screening. To allow heteroduplex formation, 5 µl of PCR products were denatured for 3 min at 95°C, followed by a gradual re-annealing as temperature was decreased from 95°C to 65°C over 30 min. The re-annealed duplexes were detected by screening on an automated dHPLC system (ProStar Helix System, Varian, CA) at flow rate of 0.45 ml/min over 8 min and through a linear acetonitrile gradient. The column mobile phase consisted of a mixture of 100 mM triethylammonium acetate (pH 7.0) (Buffer A) and 100 mM triethylammonium acetate (pH 7.0) with 25% (v/v) acetonitrile (Buffer B). The DNA fragments were detected at 260 nm. The optimum melting temperature was predicted using the melting program available online (http://insertion.stanford.edu/melt.html) and determined experimentally. PCR products were analyzed under the optimum temperature for mutation screening. To distinguish the homozygote mutant type sequence, samples showing single peak were mixed with the control DNA, under the conditions allowing heteroduplex formation, revealing a double peak, signifying the presence of the homozygote mutant sequence.

DNA sequencing. DNA sequencing of the mutated samples was later performed on an automated ABI PRISM 3100 Genetic Analyzer (PE Applied Biosystems, Foster City, CA) with BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems).

RESULTS

Out of the 76 cases, 28 (36.8%) were found to show a mutation peak on the first dHPLC screening, indicating the heterozygous of two alleles (normal and variant alleles). A second dHPLC screening of samples mixed with reference DNA revealed 7 cases with a heteroduplex peak: they were homozygous for the variant allele (9.2%). The frequency of the variant allele was 0.28.

DNA sequencing analysis showed the same substitution of A to T in intron 2, at the nucleotide position -97 upstream of exon 3 (IVS -97A>T). Here, the variant is named according to the standard nomenclature system (2).

Genotypic and allelic frequencies are shown in Table 1. The allele distribution was not significantly deviated from that expected by Hardy-Weinberg's Equilibrium ($\chi^2 = 0.5190$; $P = 0.05$). We reported this polymorphism to the dbSNP of NCBI GenBank (http://www.ncbi.nlm.nih.gov/projects/SNP) and obtained the NCBI Assay ID: ss48399641.

TABLE 1. Genotype distribution and relative allele frequencies of the IVS2 -97A>T polymorphism of the PGF2α receptor gene in 76 subjects

		Subjects
Genotype	A/A	41(53.9%)
Frequency	A/T	28 (36.8%)
	T/T	7 (9.2%)
	Total	76 subjects
Allele	A	72.3%
Frequency	T	27.7%

DISCUSSION

dHPLC has been applied to detection of mutation in several genes with high accuracy (6,13). However, its application to the screening of PGF2α receptor gene has not been reported.

The changes of the nucleotide sequences in the non-coding regions may affect the gene functions, for example, influencing the enzyme induction or activity (9,11), inducing transcription (3) or reducing in the response of fluvastatin (7). Although the SNP identified in this gene is located in the intron and may play no obvious role, the information obtained could be applied for linkage disequilibrium (LD) mapping studies. Through the studies of LD, these anonymous markers may be useful to identify the susceptibility gene without any a priori assumptions.

In conclusion, we identified a novel SNP, IVS2 -97A>T, in the PGF2α receptor gene and showed it to be a common SNP among the Malaysia patients with glaucoma. Currently two studies are underway: a study to correlate the SNP to the response to Latanoprost in glaucomatous patients and a study to clarify the frequency of the SNP in each ethnic group in Malaysia.

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