MOLECULAR GENETICS STUDY OF PROSTAGLANDIN $F_{2\alpha}$ RECEPTOR GENE IN GLAUCOMATOUS PATIENTS IN MALAYSIA

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by

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DEDICATION

Firstly, I would like to thank Allah s.w.t for giving me such a great parents, Mr. Zahary bin Hj. Daud and Mrs. Noriahti bt. Tan Abdullah. Special thanks to them for their prayer and moral support to me. For my brothers, Mohd Nizmie and Mohd Nizman, thank you for your encouragement, being a supportive brother and having a confidence in me to finish my studies. I love you all.

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LIST OF CONTENTS

LIST OF APPENDICES

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

^0C	: degree celcius
µl	: microliter
A_{260}/A_{280}	: ratio of 260 absorbance over 280 absorbance
5-FU	: 5-fluorouracil
ACG	: angle-closure glaucoma
APC	: adenomatous polyposis coli
AR	: allele ratio
ARMS	: amplification refractory mutation system
ASP	: allele specific PCR
bp	: base pair
BRCA	: breast cancer
Buffer AL	: Lysis Buffer
Buffer AW1	: Wash Buffer 1
Buffer AW2	: Wash Buffer 2
Buffer AE	: Elution Buffer
cAMP	: Cyclic Adenine Mono-phosphate
CFTR	: Cystic fibrosis transmembrane conductance regulator
CME	: cystoid macular edema
COX	: cyclooxygenase
ddH_2O	: deionized distilled water
dsDNA	: double-stranded DNA
DGGE	: denaturing gradient gel electrophoresis
dHPLC	: denaturing High Performance Liquid Chromatography

DNA	: deoxyribonucleic acid
dNTPs	: dinucleotide triphosphate
DPD	: dihydropirimidine dehydrogenase
ECM	: extracellular cell matrix
EDTA	: ethylenediamine tetraacetic acid
FBN1	: Fibrillin 1
FDA	: Food and Drug Administration
GALT	: Galactose-1-phosphate uridyl transferase
GJB2	: Gap junction B2
HMGCR	: 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors
IOP	: intraocular pressure
JNK	: c-*Jun* N-terminal kinase
LB	: Lithium Boric Acid buffer
LMNA	: Lamin A/C
LOH	: loss of heterozygosity
LQTS	: Long QT syndrome
MAPkinase	: Mitogen-activated protein kinase
MEK	: Mitogen-activated protein kinase kinase
$MgCl_2$	: magnesium chloride
min	: minute
ml	: milliliter
mM	: millimolar
MMP	: matrix metalloproteinase
MS	: multiple sclerosis
NCBI	: National Center for Biotechnology Information

ng/µl : nanogram per microliter

nm : nanometer

NTG : normal tension glaucoma

OAG : open angle glaucoma

OHT : ocular hypertension

OLA : oligonucleotide ligation assay

PCR : Polymerase chain reaction

PI3K : PI3 Kinase

PIIP : prostaglandin induced iris pigmentation

PKC : protein kinase C

PLC : phospholipase C

POAG : primary open angle glaucoma

PTEN : phosphatase and tensin homolog

QRT-PCR : Quantitative reverse-transcription polymerase chain reaction

RET : rearranged transforming

RFLP : restriction fragment length polymorphism

RNA : ribonucleic acid

rpm : round per minute

RR : relative risk

SNP : single nucleotide polymorphism

SSCP : single-strand conformation polymorphism

ssDNA : single-stranded DNA

STR : simple tandem repeat

Taq : *Thermus aquaticus*

TEAA : triethylammonium acetate

TPMT : thiopurine S-methyltransferase

TSC : tuberous sclerosis

Tx : thromboxane

U : unit

UV : ultra violet

V : voltage

VNTR : variable number of tandem repeat

KAJIAN GENETIK MOLEKUL GEN RESEPTOR PROSTAGLANDIN $F_{2\alpha}$ DI KALANGAN PESAKIT GLAUKOMA DI MALAYSIA.

ABSTRAK

Kesan sampingan yang berpunca daripada dadah antiglaukoma topikal iaitu Latanoprost telah banyak dilaporkan. Keberkesanan dadah ini mungkin berbeza bergantung kepada setiap individu. Oleh itu, hipotesisnya ialah sebarang perubahan yang berlaku pada reseptor prostanoid (FP) iaitu reseptor di mana dadah tersebut akan bertindak, mungkin bertanggungjawab dalam mempengaruhi sebarang kepelbagaian keberkesanan dadah tersebut dan juga kesan sampingannya. Matlamat kajian ini ialah untuk mengenalpasti jenis serta frekuensi polimorfisma yang terdapat pada reseptor prostanoid (FP) di kalangan rakyat Malaysia dan kesannya kepada pesakit glaukoma. Tindakbalas berantai polimerase (PCR) akan mengamplifikasi ke semua ekson gen reseptor Prostaglandin $F_{2\alpha}$. Variasi atau polimorfisma di dalam gen reseptor Prostaglandin $F_{2\alpha}$ akan disaring dengan menggunakan teknik kromatografi cecair denaturasi berprestasi tinggi (dHPLC). Mutasi yang dijumpai oleh dHPLC akan disahkan dengan teknik penjujukan DNA. Pada masa yang sama, tekanan intraokular (IOP) dan sebarang kesan sampingan pada setiap pesakit akan diawasi dalam tempoh 3 bulan. Mutasi telah dijumpai di dalam 2 daripada 12 bahagian yang mengamplifikasi keempat-empat ekson gen reseptor Prostaglandin $F_{2\alpha}$. Mutasi baru iaitu IVS3 -97A>T telah dijumpai pada 34 orang pesakit dan 32 orang kumpulan kawalan dan satu lagi mutasi iaitu EX4 1209A>G telah dijumpai pada 23 orang pesakit dan 27 orang kumpulan kawalan setelah 160 orang pesakit dan kumpulan kawalan disaring. Kedua-dua polimorfisma nukleotida tunggal (SNP) tersebut yang dijumpai di dalam gen reseptor Prostaglandin $F_{2\alpha}$ secara statistiknya tidak

mempunyai sebarang kaitan dengan glaukoma dan kesan penurunan IOP di kalangan pesakit glaukoma yang menerima latanoprost topikal. Oleh itu, kami percaya bahawa bahagian-bahagian lain di dalam gen tersebut penting untuk disaring, selain melibatkan saiz sampel yang lebih besar, penglibatan pelbagai institusi serta kajian yang lebih lama mungkin diperlukan untuk mengesahkan hubungan di antara polimorfisma di dalam gen reseptor prostaglandin $F_{2\alpha}$ dengan kepelbagaian tindak balas pesakit terhadap latanoprost.

MOLECULAR GENETICS STUDY OF PROSTAGLANDIN $F_{2\alpha}$ RECEPTOR GENE IN GLAUCOMATOUS PATIENTS IN MALAYSIA

ABSTRACT

The adverse reaction that arises from topical antiglaucoma drug namely, Latanoprost, has already been reported. The effectiveness of the drug may vary according to individual. We hypothesize that any changes in the human prostanoid (FP) receptor, the receptor to which the drug acts, could be responsible for the variation in the effectiveness of the drug as well as the adverse reaction. The objective of this study was to identify the types and frequencies of prostaglandin $F_{2\alpha}$ receptor gene polymorphisms among the Malaysian population and its implication to the glaucoma patients. Polymerase Chain Reaction (PCR) was used to amplify all the four exons of prostaglandin $F_{2\alpha}$ receptor gene. The polymorphisms of prostaglandin $F_{2\alpha}$ receptor gene was screened by using denaturing High Performance Liquid Chromatography (dHPLC). Any mutations detected by dHPLC were then confirmed by DNA sequencing. Intraocular pressure (IOP) and any side effects for each of the patients were monitored within three months post initiation of latanoprost treatment. Out of the twelve regions that amplified from the four exons of prostaglandin $F_{2\alpha}$ receptor gene, two SNPs were found. A novel SNP IVS3-97 A>T was found in 34 patients and 32 controls after 160 glaucoma patients and controls were screened. Another SNP, EX4 1209 A>G was identified in 23 patients and 27 controls among the study subjects. However, both of these SNPs which were found within the prostaglandin $F_{2\alpha}$ receptor gene did not show any statistically significant association either with glaucoma susceptibility or IOP lowering effect in glaucoma patients who received topical latanoprost. Therefore, we believed that larger coverage of

gene to be screened, larger sample size, multicenter and longer period of study is perhaps needed to confirm the possible association between polymorphisms of prostaglandin $F_{2\alpha}$ receptor gene and the variation of responsiveness of patients to the latanoprost.

CHAPTER 1

INTRODUCTION

1.1 Glaucoma

Glaucoma is defined as a progressive optic neuropathy with characteristic structural damage to the optic nerve, which results in specific visual field defects (Quigley, 1993, Kroese *et al.,* 2003). Elevated intraocular pressure (IOP) is the only modifiable risk factor, although there are a group of patients who have typical glaucomatous damage of the visual fields and/or optic nerve heads despite the IOP being consistently maintained at 21 mmHg or less (normal tension glaucoma) (Lewis *et al.,* 1983). Glaucoma is generally divided into two major types; angle-closure glaucoma (ACG) and open angle glaucoma (OAG). It may also be further divided according to the causes (primary or secondary) (Lee, 2000).

Open angle glaucoma (OAG) is the form of glaucoma which occurs in eyes with open anterior chamber angles. This form of glaucoma is further classified into primary open angle glaucoma (POAG) and its subsets, normal tension glaucoma (NTG) and ocular hypertension (OHT). POAG is the most common form of glaucoma and mainly occurs in the older age group (older than 40 years old) (Monemi *et al.,* 2005). This type of glaucoma is usually defined by three criteria: IOP consistently above 21 mmHg in at least one eye, open or normal appearing anterior chamber angle with no apparent ocular or systemic abnormality that might account for the elevated IOP and typical

glaucomatous visual field and/or optic nerve head damage. One of its subsets, NTG, is a condition where the IOP remains within limit (less than 21mmHg) but with the occurrence of progressive optic nerve damage and visual field loss (Sowka, 2005). It is thought to be related to poor blood flow to the optic nerve, which leads to the death of cells which carry impulses from retina to the brain. The other subset, OHT, is usually associated with elevated intraocular pressure (>21 mmHg) but normal optic nerve head or visual fields (Phelps, 1977). In addition, according to Ocular Hypertension Treatment Study (OHTS), OHT was defined as IOP more than 24 mmHg and less than 32 mmHg in at least one eye, IOP more than 21 mmHg and less than 32 mmHg in the fellow eye and normal visual fields and optic disc in both eyes at the time of diagnosis (Kymes *et al.,* 2006).

In general, the decrease in peripheral vision which may eventually lead to blindness in glaucoma patients is due to the optic nerve damage at the back of the eye. This damage is mainly due to the increased pressure in the eye as a result of the excessive production of aqueous humor or improper drainage of the aqueous through the outflow facilities either by conventional outflow pathway (Trabecular meshwork-Schlemm's canal) or non-conventional outflow (uveoscleral outflow) (**Figure 1.1**) (**Figure 1.2**). Aqueous humor is produced by the ciliary body which involves the blood flow to the ciliary processes, ultrafiltration of plasma in tissue spaces of ciliary processes and secretion by ciliary epithelium (Brubaker, 1991). Then, it flows from the ciliary epithelium into the posterior chamber and then through the pupil into the anterior chamber. Subsequently, it flows out through the trabecular meshwork-Schlemm's canal or the uveoscleral outflow pathway to the systemic circulation. The elevated pressure in the eye as mentioned above, results in progressive changes in the optic nerve head and retina where it involves the axonal and non-axonal effects and retinal ganglion cells

appeared to undergo changes in neurotrophin response prior to cell death respectively (Morrison *et al.,* 2005). Thus, it may influence the relative susceptibility to IOP and explain the progressive optic nerve damage and visual field loss.

1.1.1 Prevalence of glaucoma

Primary open angle glaucoma (POAG) is one of the leading causes of irreversible visual loss in Western countries with the prevalence of 1.1% to 3.0% (Dielemans *et al.,* 1994). Based on an extensive systemic review of 111 published reports by Quigley (1996) on the prevalence of glaucoma, 66.8 million was estimated to have primary glaucoma, with 6.7 million suffering from bilateral blindness. Another report by Quigley in 2002 estimated that people suffering from bilateral blindness by OAG and ACG will be increased to 7.6 million. It is estimated that 60.5 million people will suffer from OAG and ACG by 2010, increasing to 79.6 million people by 2020, 74% of them will have OAG and Asians will represent 47% of those who having glaucoma (Quigley and Broman, 2006). Similarly, 4.5 million people with OAG and 3.9 million people with ACG is estimated to develop bilateral blindness in 2010, further increased to 5.9 and 5.3 million people in 2020 respectively (Quigley and Broman, 2006). Thus, without doubt, glaucoma has emerged as the second leading cause of blindness worldwide, after cataract (Quigley and Broman, 2006).

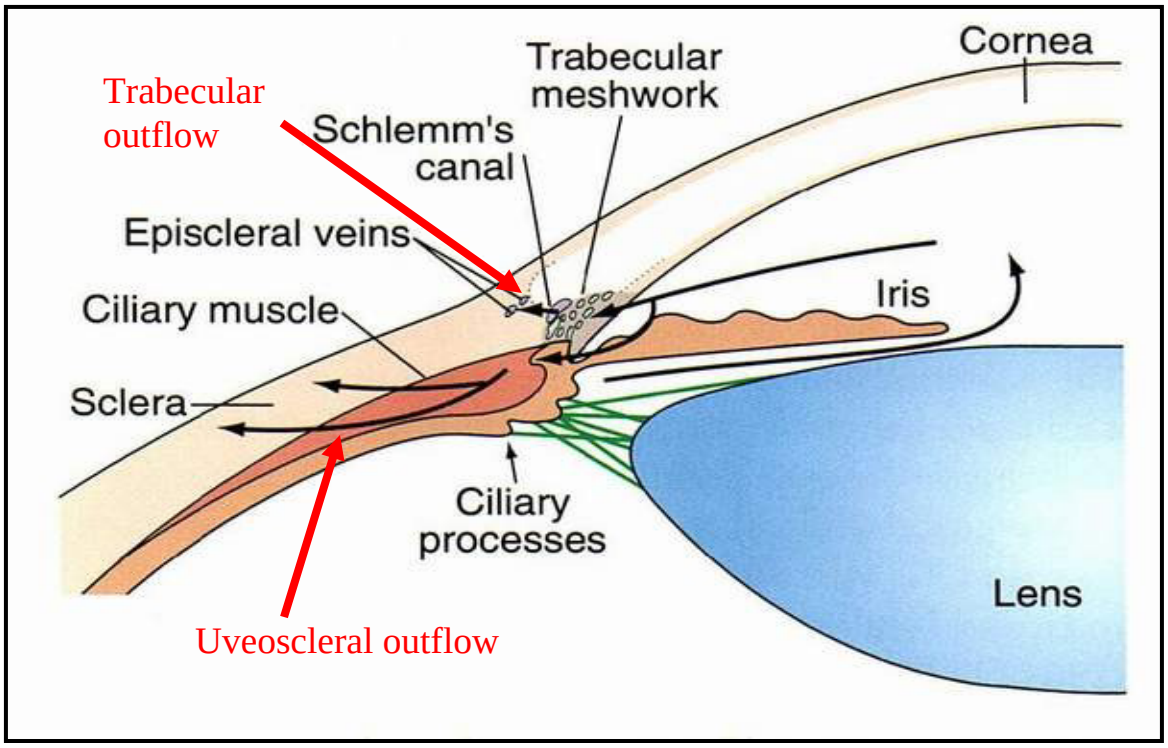

Figure 1.1: The drainage of aqueous humor either by conventional outflow pathway (Trabecular meshwork-Schlemm's canal) or non-conventional outflow (uveoscleral outflow). Adapted from: http://www.unmc.edu/dept/physiology/index.cfm

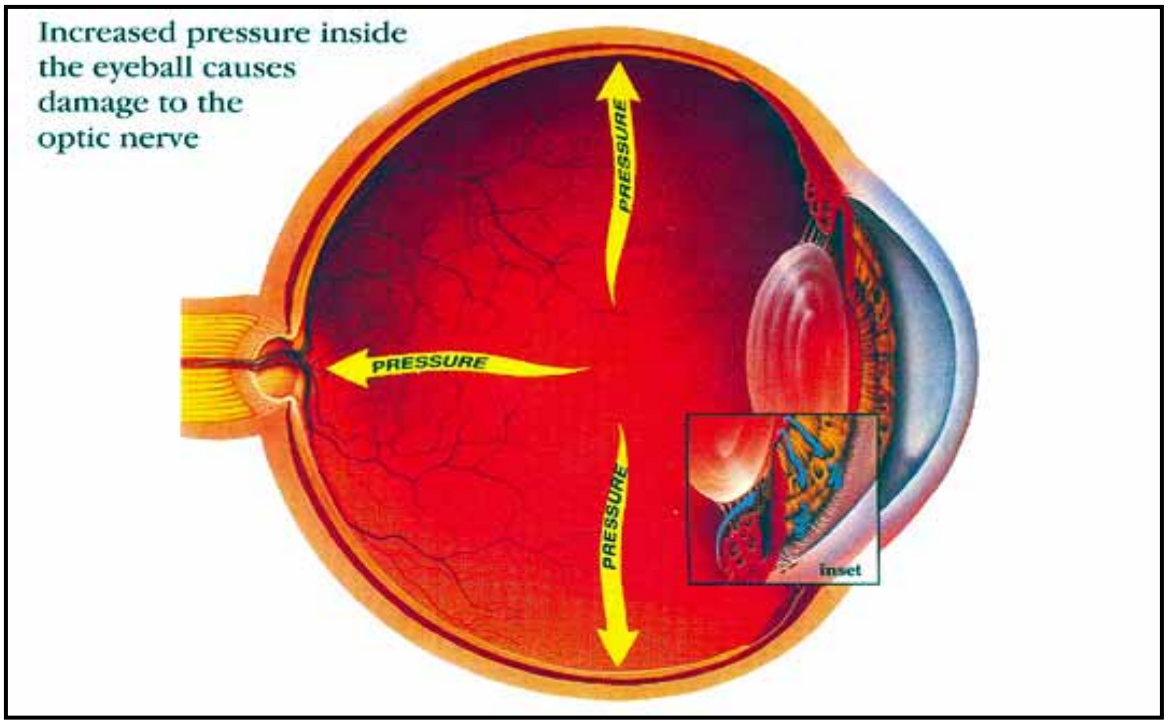

Figure 1.2: Elevated intraocular pressure ultimately will cause damage to the optic nerve at the back of the eye. Adapted from: http://www.theeyefoundation.com/glaucoma.htm

In Singapore, based on population-based cross-sectional study, glaucoma was found as a leading cause of blindness among Singaporean Chinese adults which contributed to 60.0% of bilateral blindness, with the age and gender-adjusted prevalence rates being 1.1% and 0.5% for bilateral low vision and bilateral blindness respectively (Saw *et al.,* 2004). Similar population-based cross-sectional study conducted in Thailand, showed that glaucoma was the second most common cause of severe unilateral visual loss. Primary open angle glaucoma (POAG) accounted for 67% of all glaucoma (Bourne *et al.,* 2003). They also reported that 11% of the bilateral blindness was caused by glaucoma. In Malaysia, a National Eye Survey conducted in 1996, found that cataract was still the leading cause of blindness (39%) followed by retinal disease (24%), uncorrected refractive error and corneal disease. Glaucoma was only ranked fifth as the major cause of blindness (1.8%) (Zainal *et al.,* 2002), which was mainly due to high percentage of underestimation on the prevalence of glaucoma. However, the examination was conducted by using only direct ophthalmoscope and torch light without proper glaucoma diagnostic tools such as tonometer, gonioscopy and visual field assessment.

In general, the risk of blindness from glaucoma is very high among patients in the developing countries (Chen, 2004). Late presentation was found as the major cause of blindness in 29% to 41% of glaucoma patients (Kwon *et al.,* 2001, Oliver *et al.,* 2002, Chieng *et al.,* 2005). Poor awareness of the disease is closely related to the late presentation. Famously known as the silent thief of vision, OAG is asymptomatic, patient only become aware once the disease is in advanced stage. According to Hugh Taylor and Jill Keeffe (2001), at least half of people with glaucoma were under diagnosed and consequently, not treated. In spite of better awareness, the problems are not better in the developed countries. A survey on German population revealed that 75% of the

respondents have heard about glaucoma but only 8% correctly defined glaucoma (Pfeiffer *et al.,* 2002). Almost similar findings were also reported from Hong Kong with fewer numbers even aware of the mode of treatment which include drugs, laser and surgery (Lau *et al.,* 2002).

1.1.2 Management of glaucoma

Management of glaucoma ranges from the safest and the least invasive method to those that expose the patients to greater risk and are more invasive. Basically, glaucoma therapy involves medical therapy, laser treatment and filtrating surgery (trabeculectomy), which aim at the reduction of IOP; the only modifiable risk factors. Till now IOP is the only manipulative tool to prevent further nerve fiber damage as has been shown by several large, randomized control trials, where lowering the IOP has been associated with the retardation of progressive optic nerve damage (Morrison *et al.,* 1998, Heijl *et al.,* 2002, Leske *et al.,* 2003).

The goal of antiglaucoma therapy is the achievement of a targeted intraocular pressure. Target pressure is defined as the mean of an estimated IOP with treatment that is expected to prevent further glaucomatous damage (Spaeth, 2000) or as the level of intraocular pressure which is associated with minimal likelihood of visual field or optic nerve lesion or an existing lesion progression due to elevated intraocular pressure (Popovic-Suic *et al.,* 2005). For example, glaucoma therapy in ocular hypertension patients should at least achieve 20% IOP reduction from baseline in those with moderate to high risk glaucoma, based on perimetric changes; IOP should be lowered by at least 30% in early to moderate glaucoma and 40% to 50% in severe glaucoma (Schwartz and Budenz, 2004). An additional 15% of IOP reduction is targeted if there is evidence of

visual field progression (American Academy of Ophthalmology Preferred Practice Patterns Committee Glaucoma Panel, 2001). Several randomized clinical trials such as Collaborative Normal Tension Glaucoma Study (CNTGS), Advanced Glaucoma Intervention Study (AGIS), Collaborative Initial Glaucoma Study (CIGS), Ocular Hypertension Treatment Study (OHTS) and Early Manifest Glaucoma Treatment Study (EMGTS) provided evidence that above cited parameters may be useful towards the setting of initial IOP goal in glaucoma patients (Heijl *et al.,* 2002, Anderson, 2003, Feiner and Piltz-Seymour, 2003, Nouri-Mahdavi *et al.,* 2004, Kymes *et al.,* 2006). All these studies concluded that IOP reduction is very essential in preventing further progression of glaucoma.

Though there are a few options for management of IOP in glaucoma but medical treatment, mainly using topical antiglaucoma drugs, still remains the first line management of glaucoma as it is non invasive method and easy to administer. In addition, patients' compliance with medication regimen is also critical in evaluating the effectiveness of treatment. The instillation of topical antiglaucoma drugs which is easy, will improve a patient's compliance. Ocular hypotensive effects are induced by a topical antiglaucoma drug either by reducing the production of aqueous humor or by increasing the outflow of aqueous.

Before the emergence of prostaglandin derivatives as ocular hypotensive agents, there were several classes of medications available. Currently, there are at least five different classes of anti-glaucoma agents including beta blocker (timoptol, betaxolol), parasympathetic agonist (pilocarpine), carbonic anhydrase inhibitor (dorzolamide, brinzolamide), adrenergic agonists (propine, adrenaline) and alpha 2 agonist (brimonidine). Prostaglandin analogues like latanoprost, travaprost and bimatoprost are

other newly discovered class of ocular hypotensive agents. So far, only latanoprost has been approved for first line therapy (Physicians Desk Reference for Ophthalmic Medicines, 2003).

1.2 Latanoprost

1.2.1 Background

Management of glaucoma was rather difficult before 1995, since only five groups of topical antiglaucoma medications were available, namely cholinergic agonist, parasympathetic agonist, beta-blockers, alpha-2 agonist and carbonic anhydrase inhibitors. Significant efforts and advances have been achieved later in the development of topical antiglaucoma medications. Since the introduction of latanoprost in 1996, prostaglandin analogues had emerged as one of the most prescribed antiglaucoma medication among the ophthalmologists. Prostaglandin analogues (latanoprost, bimatoprost and travoprost) are the most potent pressure lowering agents (Alexander *et al.,* 2002). The prostaglandin analogues (bimatoprost 0.03% and travoprost 0.004%) were proven to be more effective in lowering the IOP than the previous first-line agent, timolol 0.5% (Netland *et al.,* 2001, Sherwood and Brandt, 2001).

Prostaglandins are autacoids lipid derivative generated by metabolism of arachidonic acid by the cyclooxygenase (COX) and prostaglandin synthase enzymes (Hata and Breyer, 2004). Prostaglandins modulate many of the physiological systems including the CNS, cardiovascular, endocrine, respiratory and immune systems. It is also generally considered to be a potent pro-inflammatory mediator (Hata and Breyer, 2004). The expression of one of the COX enzyme, COX-2, mainly depends on the response to

inflammatory cytokines and bacterial lipopolysaccharide (Masferrer and Kulkarni, 1997, Hata and Breyer, 2004). Therefore, those prostaglandins that are generated via COX-2 are responsible for the inflammatory effects. Studies also demonstrated that there was a significant increase in prostaglandin in aqueous humor and tear fluid in clinical ocular inflammatory conditions and diseases (Kulkarni and Mancino, 1993, Hata and Breyer, 1997). The production of inflammatory prostaglandin in ocular tissues also resulted from the COX-2 expression since this enzyme plays a major role in the production of prostaglandin in the inflammatory cascade.

Topical latanoprost 0.005% (Xalatan, Pfizer), $PGF_{2\alpha}$ analogue, a selective agonist of the FP receptor, is well tolerated and has become one of the most commonly used drug to treat glaucoma and ocular hypertension (Alm, 1998). Latanoprost (code name PhXA41; 13,14-dihydro-17-phenyl-18,19,20-trinor-$PGF_{2\alpha}$-isopropyl ester) is a prodrug with a phenyl ring substituted for carbon 18-20 in the omega chain and it have a saturated double bond between carbons 13 and 14 (Stjernschantz and Resul, 1992) (**Figure 1.3**). It is rapidly hydrolysed in the cornea and blood to its primary metabolite, latanoprost acid following topical instillation into the eye (Sjoquist and Stjernschantz, 2002).

In spite of an excellent pressure lowering agent, the exact mechanism of action of latanoprost is not really established. It is believed that ocular hypotensive effect is achieved by increasing the aqueous humor outflow through both the trabecular route (via Schlemm's canal and episcleral veins) and the uveoscleral (ciliary muscle) pathway. The precise mechanism of prostaglandin analogues in achieving pressure lowering effect had created various postulations and debates. One of the well accepted postulations is $PGF_{2\alpha}$ analogue reduces the IOP by increasing the uveoscleral outflow of aqueous

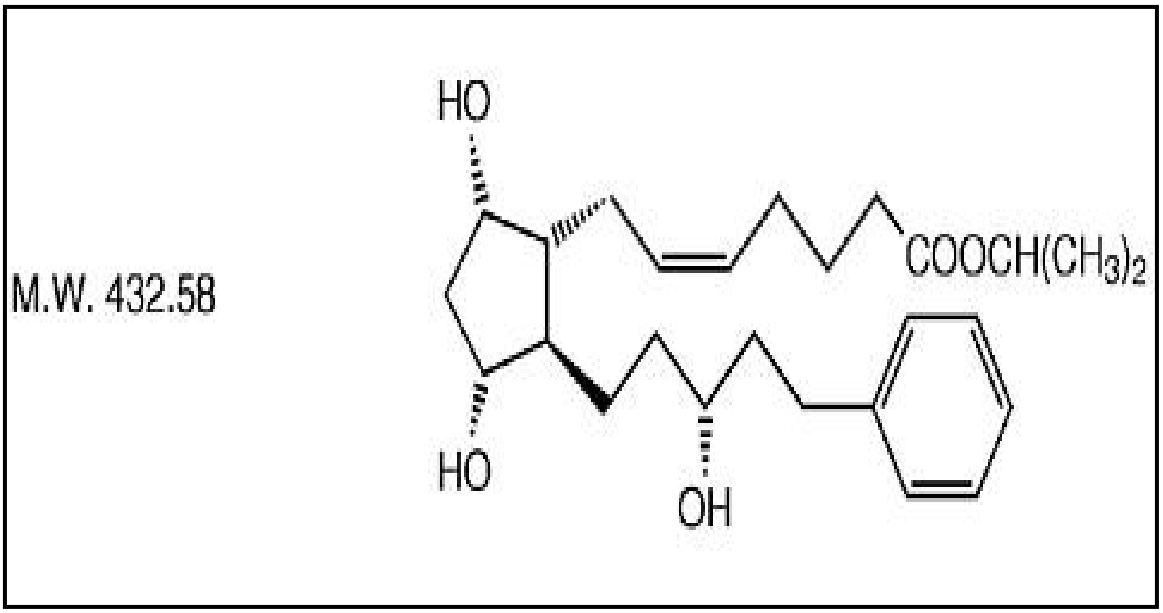

Figure 1.3: Chemical structures of latanoprost which consists of two long carbon chains (alpha and omega). Adapted from: http://www.chem-online.org/generic-pharmaceutical/latanoprost.htm

humor possibly by reducing the resistance between the ciliary muscles, through the effect on the extracellular matrix (ECM) (Kunapuli *et al.,* 1997).

Matrix metalloproteinase (MMP) is a family of neutral, zinc-dependent enzymes that can hydrolyze specific peptide sequences found in ECM structural proteins (Birkedal-Hansen *et al.,* 1993). There is evidence that exposure to $PGF_{2\alpha}$, 11-deoxy-PGE_1 or latanoprost acid (the active form of latanoprost), increased the secretion of MMP-1, MMP-2, MMP-3 and MMP-9 by human ciliary smooth muscle cells (Weinreb *et al.,* 1997). For example, MMP-1 efficiently targets a specific site found in the fibrillar collagen types I and III. MMP-3 efficiently cleaves specific sites found in collagen types IV, IX, XI as well as in fibronectin and aggrecan core protein. MMP-2 cleaves sites in collagen IV, V, VII and X. MMP-9 cleaves sites in collagen IV and V. Hence, these MMPs may lead to alterations of numerous ECM components within the ciliary muscle (Weinreb *et al.,* 1997, Ocklind, 1998, Weinreb *et al.,* 2002).

The reduction of ciliary muscle ECM within the extracellular spaces among the ciliary muscle fiber bundles where aqueous humor passes through the uveoscleral outflow, could reduce the hydrolic resistance to aqueous movement and increases the uveoscleral outflow (Lindsey *et al.,* 1997, Weinreb *et al.,* 2002) (**Figure 1.4**). Another report suggested that $PGF_{2\alpha}$ induced vasodilation in the ciliary body, and enhances the uveoscleral outflow by tissue expansion (Bill, 1989).

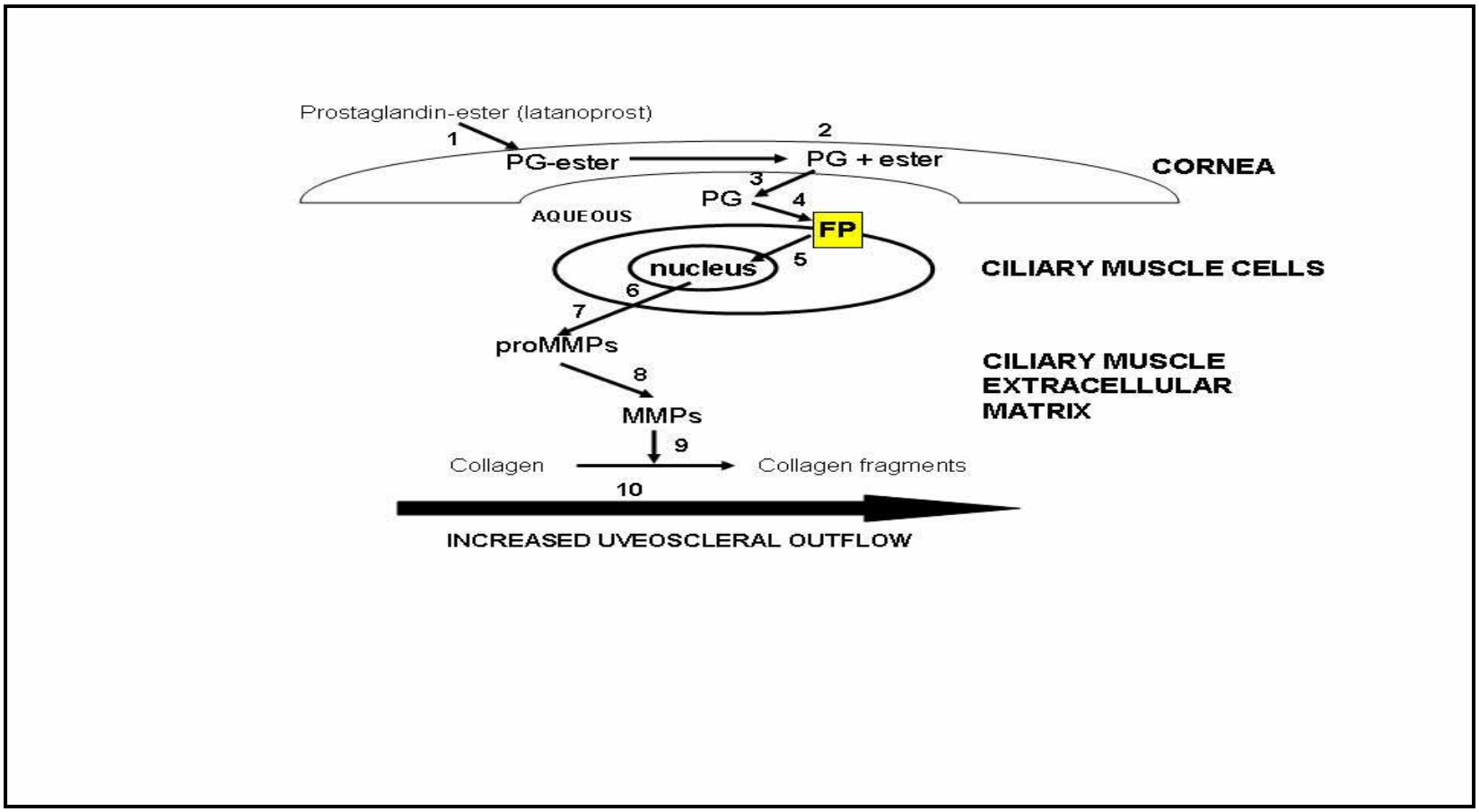

Figure 1.4: The diagram shows the possible mechanism of latanoprost-induced uveoscleral outflow. Topical latanoprost absorbed into the cornea **(1)** where it is converted to free prostaglandin (PG) **(2)**. It passed into the aqueous **(3)** and binds to the FP receptor on ciliary muscle cell surface **(4)**. This binding starts a signal cascade leading to formation of AP1-transcription factor in nucleus **(5)** which induces the transcription of MMP genes **(6)**. Then, it translated into proMMPs and secreted into extracellular space around the ciliary muscle fibers **(7)**. Proteolytic truncation induces activation of MMPs **(8)** which help to degrade the extracellular matrix components (collagens) **(9)**. The reduction of collagens inside the uveoscleral outflow pathway decreased the hydraulic resistance and facilitates the outflow **(10)**.

Whatever the postulations, the evidence concentrate on relaxation of the ciliary muscle as the most likely mechanism of latanoprost-enhanced uveoscleral outflow. However, the prostanoid FP receptor needs to couple to Gq protein in order to activate the phospholipase C, IP3 generation and calcium mobilization, which is associated with contraction of smooth muscle (Coleman *et al.,* 1994).

1.2.2 Intraocular Pressure (IOP) Lowering Effect of Latanoprost

1.2.2.1 Latanoprost as monotherapy treatment

Latanoprost has been proven to be effective in reducing the IOP either as a monotherapy or adjunctive therapy in patients who failed to achieve desirable IOP with their current glaucoma treatment regimen (Rulo *et al.,* 1994, Alm and Stjernschantz, 1995, Mishima *et al.,* 1996, Aquino and Lat-Luna, 1999, O'Donoghue, 2000, Bron *et al.,* 2001). In addition, many randomized clinical trials conducted on patients with open-angle or ocular hypertension, revealed that monotherapy with latanoprost reduces IOP levels by 20% to 40% at 1 and 12 months of treatment (Alm and Stjernschantz, 1995, Camras *et al.,* 1996, Mishima *et al.,* 1996, Watson and Stjernschantz, 1996, Aquino and Lat-Luna, 1999). The maximum ocular hypotensive effect can be achieved within 8 to 12 hours after instillation of once daily topical dose of latanoprost 0.005% to both healthy individuals and open-angle glaucoma and ocular hypertension patients in Japan and Europe (Hotehama *et al.,* 1993, Racz *et al.,* 1996, Mishima *et al.,* 1997). In addition, latanoprost was also found to be able to provide uniform circadian (around-the-clock) IOP reduction (Racz *et al.,* 1996). The magnitude of IOP reduction was found to be essentially identical during both daytime and nighttime hours (Mishima *et al.,* 1997).

1.2.2.2 Comparison with other topical antiglaucoma drugs as monotherapy

Latanoprost has proven to be more effective in reducing the IOP when compared to other classes of topical antiglaucoma drugs such as beta blocker (timolol), carbonic anhydrase inhibitor (dorzolamide) and alpha-2 agonist (brimonidine) as monotherapy treatment (Camras, 1996, Einarson *et al.,* 2000, O'Donoghue, 2000). Latanoprost 0.005% once daily was significantly more effective than timolol 0.5% twice daily at 3 to 6 months of treatment (Alm and Stjernschantz, 1995, Camras *et al.,* 1996, Mishima *et al.,* 1996, Aquino and Lat-Luna, 1999). It reduces mean baseline diurnal IOP by 6.2 to 11.1 mmHg (27% to 39%) compared to 4.4 to 9.1 mmHg reduction by topical timolol (19% to 33%). The pressure lowering effect of latanoprost is not only greater but more consistent at daytime and nighttime compared to timolol (Larsson, 2001). A randomized crossover trial studies on latanoprost and timolol found significantly greater mean IOP reduction both during daytime and nighttime hours with latanoprost ($p < 0.001$) (Orzalesi *et al.,* 2000, Larsson, 2001).

Latanoprost 0.005% once daily was also found to be significantly effective than dorzolamide 2% three times daily (O'Donoghue, 2000). A meta analysis involving nine comparative studies on glaucoma patients who received latanoprost and brimonidine, showed latanoprost providing a greater IOP reduction compared to brimonidine after 3 months (33% to 26% of IOP reduction by brimonidine) and 6 months of treatment (32% to 25% of IOP reduction by brimonidine) (Einarson *et al.,* 2000). In spite of various evidences of better IOP lowering effect, several randomized clinical trials found that latanoprost 0.005% have no clinically significant difference in the ability of IOP-lowering effect at 8.00 am, the time of peak effect with bimatoprost 0.03% or travoprost 0.004% (Gandolfi *et al.,* 2001, Netland *et al.,* 2001, Walters *et al.,* 2001, Parrish *et al.,* 2003).

However, Parrish *et al.,* (2003) who conducted a 12-week, randomized, masked-evaluator, multi-center study on patients with open-angle glaucoma and ocular hypertension on once daily treatment with latanoprost 0.005%, bimatoprost 0.03% and travoprost 0.004% and found that baseline mean 8.00 am IOP were similar among the treatment groups with significant reduction from baseline with each treatment after 12 weeks. This 12 weeks study demonstrated that latanoprost, bimatoprost and travoprost are equally potent IOP lowering agents that are generally well tolerated systemically.

1.2.2.3 Adjunctive therapy

Latanoprost is well known to be an effective pressure lowering agent either as monotherapy or adjunctive therapy compared to the other classes of antiglaucoma drugs. A study conducted in France found that there was a good effect on IOP reduction achieved when adding latanoprost to timolol in patients with inadequate IOP control with timolol in open angle glaucoma or ocular hypertension (Bron *et al.,* 2001). It also proved to be a good IOP lowering agent compared to the other classes of antiglaucoma drugs when used as adjunctive therapy to timolol. There was similar findings found by Italian and German Latanoprost Study Group where greater IOP reduction in diurnal IOP was achieved after adding latanoprost compared to pilocarpine in patients whom IOP was inadequately controlled with timolol alone (Bucci, 1999, Diestelhorst, 2000). The IOP lowering effect of latanoprost also was found to be superior to dorzolamide and brimonidine when added to patients who was previously treated with timolol (Petounis *et al.,* 2001, Simmons and Earl, 2002).

Rulo *et al.,* (1997) found that the mean IOP of patients with elevated IOP treated with acetazolamide was further reduced after topical administration of latanoprost. In a

preliminary study involving untreated ocular hypertensive patients who was initially treated with 2% pilocarpine three times a day for three days, achieved further additional IOP reduction with topical latanoprost (Villumsen and Alm, 1992). In addition, another study demonstrated the reduction of IOP by an additional 14% after latanoprost was added to pilocarpine in patients with elevated IOP (Fristrom and Nilsson, 1993).

1.2.2.4 Latanoprost non-responder

Even though latanoprost has been regarded as a powerful IOP lowering agent, there are patients who demonstrated failure to achieve IOP reduction, also known as non-responders. The reported incidence of latanoprost non responders ranged from 12% to 41% in American and European population (DuBiner *et al.,* 2001, Scherer, 2002, Williams, 2002). However, the definition of non-responders which varies from one study to another, lead to difficult in truly assessing the response. For example, Scherer, (2002) defined the non responders as patients who achieved IOP reduction less than 20% of baseline or reduction of less than 5 mmHg (Scherer, 2002). While Williams, (2002), categorized patients with IOP reduction of less than 3 mmHg after 4 weeks of treatment as non responders and DuBiner *et al.,* (2001) defined it as less than 20% IOP reduction at 3 months past treatment. Noecker *et al.,* (2003) compared the IOP lowering efficacy and safety of topical bimatoprost 0.03% with latanoprost 0.005% found that 17.6% to 25% of patients who was treated with latanoprost achieved less than 15% IOP reduction. Netland *et al.,* (2001) defined non responder as those who achieved less than 3 mmHg of IOP reduction at 20 hours after dose and found that 13.5% non responder rate in their study. Ikeda *et al.,* (2006) investigated the incidence and clinical profiles of latanoprost non responders in Japanese population and found that after 12 months of treatment, the incidence of latanoprost non responders was 31.8%, which higher than American and

European patients. A study conducted by Aung *et al.,* (2001), compared the intraocular pressure lowering effect and side effect of latanoprost 0.005% with unoprostone 0.12% among Singaporean patients with POAG and ocular hypertension found that 10.3% to 14.8% of patients show less than 15% of IOP reduction from baseline.

A big question for such variation of response to latanoprost still remains. Ikeda *et al.,* (2006) postulated that the delivery of inadequate concentrations of the drug to the intraocular system may contribute to the high rate of latanoprost non responder (Ikeda *et al.,* 2006). Racial differences and ethnic background is thought to play a role in the differences of response among various populations. Some even postulated that the differences of pigmentation of iris, hair and dermis among population may also contribute to the rate of latanoprost non responder (Netland *et al.,* 2001, Ikeda *et al.,* 2006). Genetic factors were also postulated to be involved and play a role in the variation of responsiveness to latanoprost (Ikeda *et al.,* 2006).

1.2.3 Adverse Effects of Latanoprost

1.2.3.1 Conjunctival hyperemia

Even though it has been proven to be a good drug in reducing IOP, adverse effects such as hyperemia and iris pigmentation were reported (Patel and Spencer, 1996, Alm *et al.,* 1997). Most of the adverse events are mild and reversible when treatment is discontinued. One of the most common side effects of latanoprost is conjunctival hyperemia (Racz *et al.,* 1993, Alm *et al.,* 1995, Camras, 1996). This adverse effect could become a concerning issue among the ophthalmologists because it may compromise the outcome of filtration surgery and may represent a cosmetic problem to the patients

leading to non-compliance (Feldman, 2003). Studies conducted in Scandinavia, United Kingdom and United States of America reported that 4%, 15% and 5% respectively of the occurrence of mild conjunctival hyperemia as ocular side effect on latanoprost treatment (Alm *et al.,* 1997). The incidence of ocular adverse events in short term trials are almost similar between latanoprost and bimatoprost (DuBiner *et al.,* 2001). However, in another study by Gandolfi *et al.,* (2001), bimatoprost and travoprost shows substantially higher rates of ocular adverse reactions especially conjunctival hyperemia. According to Zhang *et al.,* (2001), the risk for developing hyperemia after receiving latanoprost treatment was over twice that seen in timolol.

The development of conjunctival hyperemia is believed to be due to secondary, unrelated mechanism (Feldman, 2003). In fact, variation in the incidence of conjunctival hyperemia is believed to occur due to the differences in chemical structures of prostaglandin derivatives (Feldman, 2003). The molecular structure of other prostaglandin analogues such as travoprost and bimatoprost differ from latanoprost, with the presence of saturated double bond at the C_{13}-C_{14} position of the molecule. The double bond at this particular position is believed to enhance the occurrence of conjunctival hyperemia as the adverse effect (Stjernschantz, 2001). Resul and Stjernschantz, (1993) proposed another theory, that this could be caused by a vasodilation related to the release of nitric oxide resulting from the excessive production of nitric oxide synthase induced by prostaglandin analogues (Resul and Stjernschantz, 1993). However, the precise mechanism on the release of nitric oxide remains unknown (Astin *et al.,* 1994, Stewart *et al.,* 2003).

1.2.3.2 Iris hyperpigmentation

Iris hyperpigmentation has been one of the most intriguing adverse effects of latanoprost. Latanoprost has been reported to induce iris hyperpigmentation in humans and monkeys (Alm *et al.,* 1997, Selen *et al.,* 1997, Albert *et al.,* 2000). The iridial pigment changes as well as periocular skin pigmentation and eyelash hypertrichosis, later were regarded as the side effect of latanoprost and other prostaglandin analogues in various clinical studies (Alm and Stjernschantz, 1995, Camras, 1996, Watson and Stjernschantz, 1996, Netland *et al.,* 2001, Parrish *et al.,* 2003). In human, increase in iris hyperpigmentation was reported to be 5 to 15% of the patients who were treated with topical latanoprost where the highest number of cases occurring in patients with 'hazel' or heterochromatic eye colour (Wistrand *et al.,* 1997). In contrast, Alm *et al.,* (1995) reported that incidence was found to be as high as 69.7% with higher risk in irides of mixed colour than in blue, grey or brown irides. In addition, a retrospective study conducted in Japan revealed that 42% of patients had increased iris hyperpigmentation induced by latanoprost after 12-month follow-up (Hara, 2001). On the other hand, Chiba *et al.,* (2004) conducted a prospective study of iridial pigmentation as a result of treatment with latanoprost and found that there was definite increase in iridial pigmentation after 6-months and 12-months of treatment.

The precise mechanism of prostaglandin-induced iris hyperpigmentation (PIIP) remains to be elucidated. Among the possible mechanisms that have been focused by many of the researchers include the regulation of melanogenesis processes such as the enzyme tyrosinase which is involved in the synthesis of melanin, production of PGE_2 or sympathetic system innervation (Drago *et al.,* 1999, Dutkiewicz *et al.,* 2000, Hu *et al.,* 2000, Stjernschantz, 2001). Latanoprost was found to induce the melanin production

which involved the tyrosinase activity via a non-cAMP-dependent pathway (Hu *et al.,* 2000). It was also found to affect tyrosinase at the gene transcription level in iridial melanocytes as observed in treated and control cynomolgus monkeys (Stjernschantz *et al.,* 2000).

It is also postulated that latanoprost possess a potential to exert a melanogenic effect via a melanocytic FP receptors (Ocklind *et al.,* 1996). While unoprostone, the other prostaglandin analogue with low affinity for the FP receptor, enhances the tyrosine activity, which was also found to be associated with the increase of iris hyperpigmentation in clinical trials (Chiba *et al.,* 2003, Zhan *et al.,* 2003). This evidence may suggest that the stimulation of FP receptor in melanogenesis is questionable. On the other hand, latanoprost was also found to be consistently inducing the in vitro production of PGE_2 in iridial melanocytes (Stjernschantz, 2001). In another study, increased melanogenesis in cultured iridial melanocytes was also found to be coincided with increased PGE_2 production (Bergh *et al.,* 2002). The increase of PGE_2 production in melanogenesis may be mediated by cyclooxygenase-2 enzyme (Bhattacharya *et al.,* 1998).

1.2.3.3 Hypertrichosis

Recently, latanoprost has been recognized as a drug which is capable of inducing hypertrichosis involving eyelashes, adjacent adnexal hair and vellus hair of the skin (Johnstone, 1997, Wand, 1997). Latanoprost treatment was found to increase thickness, length and curvature of the lashes as well as resulted in full growth of eyelashes in a patient with previous loss of eyelashes due to alopecia (Johnstone, 1998, Mansberger and Cioffi, 2000). Glaucoma patients who were treated with latanoprost unilaterally,

showed evidence of hypertrichosis in the treated eye (Johnstone, 1997). However, latanoprost-induced eyelash hypertrichosis was also found to be reversed at 8 months after the medication was discontinued (O'Toole *et al.,* 2001).

To date, the mechanism by which latanoprost causes eyelash hypertrichosis is still not fully understood. Prostaglandins are important stimulants of melanogenesis and the FP receptor which latanoprost binds has been localized in majority of ocular tissues as well as the hair follicle (Woodward *et al.,* 1997, Stjernschantz, 2001). The pigmented melanosomes in the bulb of hair follicle also can be transferred to hair keratinocytes (Wand, 1997).

1.2.3.4 Other side effects of latanoprost

Red eye or conjunctival hyperemia is another important side effect of latanoprost. Since prostaglandins are mediators of topical inflammation in the eye, it is not surprising the synthetic prostaglandins or ocular hypotensive lipids induce inflammatory processes or cause irritating reactions. Compared to other prostaglandin analogue, latanoprost appeared to be the least reported ocular irritation (Larsson, 2001). In addition, cystoid macular edema (CME) was also reported in several cases (Miyake *et al.,* 1999, Netland *et al.,* 2001). However, there was no study that was able to estimate the risk of developing cystoid macular edema after administration of latanoprost. The risk appears to be increased if the natural lens is missing or the posterior capsule of the eye is destructed. However, latanoprost induced cystoid macular edema is a transient effect, which resolve after discontinuation of the treatment (Larsson *et al.,* 2002).

In contrast to topical timolol, systemic adverse reactions are infrequent with latanoprost therapy, perhaps due to the fast degradation of prostaglandin (Patel and Spencer, 1996). It is due to the presence of enzymes which degrade the naturally occurring prostaglandins not far from the site that they are produced. Clinical studies of latanoprost also found no significant effects on peak expiratory flow, forced expiratory volume in one second, forced ventilatory capacity, asthma symptoms or asthma medication requirements (Hedner *et al.,* 1999, Waldock *et al.,* 2000). Even though there are several adverse reactions of latanoprost, but not all the patients will experience all the side effects. The postulation is that the effectiveness of the drugs varies according to individuals, and could be attributed to any changes in the receptor which may be responsible for the variation of effectiveness of the drug as well as the adverse reaction. Variations in the gene coding for the receptors are therefore believed to be also responsible for the variations in the receptors. This branch of science that deals with the relation of genetic factors to variation in response to drugs is called pharmacogenetics.

1.3 Pharmacogenetics

The word pharmacogenetics which usually has been used interchangeably with pharmacogenomics can be defined as the study of the impact of genetic variation on the efficacy and toxicity of drugs, or the study of how genetic makeup determines the response to a therapeutic intervention (Zilfalil, 2005). The development of pharmacogenetics over the years remained slow since family studies were difficult and a direct DNA study was not yet possible (Motulsky and Qi, 2006). Pharmacogenetics also suffered from the lack of integration into clinical practice. There were several reasons mainly due to unmet need for education at medical schools and the lack of awareness

about the impact of genetic medicine on healthcare in the community (Frueh and Gurwitz, 2004).

Inter-individual variability of drug response poses a major problem in clinical practice with some patients do not response favorably or suffered severe adverse drug reactions. A meta-analysis of 39 prospective studies from United States hospitals suggests that adverse drug reactions are responsible for about 2.2 million hospitalizations and 100,000 deaths per year (Lazarou *et al.,* 1998). The high frequencies of adverse drug reactions cases and its association with high mortality and morbidity rates are now becoming a major health policy concern worldwide.

Pharmacogenetic studies have shown that polymorphisms of drug metabolizing enzymes, transporters and receptors contribute to variable drug response (Evans and Relling, 1999, Evans and Johnson, 2001). There were also numerous other factors that contribute to variable drug response including age, sex, body weight, nutrition, organ function, infections and co-medications. Although these non-genetic factors can be very important, but the inherited differences in the metabolism and disposition of drugs, and genetic polymorphisms in the targets of drug therapy (receptors) and drug transporter can have a major influence on the efficacy and toxicity of many medications. For example, the missense mutation and polymorphic CAG microsatellite in the gene encoding androgen receptor has reversed its effect and contribute its failure to response to antiandrogenic therapy that usually used in the therapy of prostate cancer (Schoenberg *et al.,* 1994). Single nucleotide polymorphisms resulted in changes of the amino acids 16 and 27 of the beta2-adrenergic receptor found to alter the responsiveness of anti-asthmatic chemotherapy among the asthmatic patients (Drysdale *et al.,* 2000).