PROSTANOID (FP) RECEPTOR POLYMORPHISMS:

THE ASSOCIATION OF NOVEL SINGLE NUCLEOTIDE POLYMORPHISM WITH THE RESPONSIVENESS OF GLAUCOMA PATIENTS TO TOPICAL LATANOPROST

BY

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DISCLAIMER

I hereby certify that the work in this dissertation is done on my own.

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ABSTRAK

Pengenalan: Kepelbagaian respons pesakit glaukoma terhadap ubat titis mata latanoprost bukannya isu yang baru, kebanyakan penyelidik berpendapat ia melibatkan faktor kaum, rejim rawatan dan mungkin juga akibat polimorfisma gen.

Objektif: Kajian ini bertujuan mengenalpasti kewujudan polimorfisma gen pada PTGFR dan peranannya ke atas keberkesanan ubat titis mata latanoprost di kalangan pesakit glaukoma.

Metodologi: Pesakit-pesakit glaukoma yang dirawat dengan ubat titis mata latanoprost diperiksa dan disusuli selama 6 bulan. Corak tekanan mata dikaji dan perbezaan respons pesakit dikenalpasti. Polimorfisma gen pada bahagian ekson gen PTGFR dikenalpasti di kalangan pesakit dan juga kontrol dengan menggunakan kaedah 'dHPLC' (denaturing high performance lipid chromatography). Polimorfisma yang ditemui itu akan dikaitkan dengan corak penurunan tekanan mata dan perbezaan respons pesakit.

Keputusan: Seramai 76 pesakit glaukoma dan kontrol telah disaring dan tiada variasi ekson yang ditemui. Walaubagaimanapun, satu polimorfisma nukleotida tunggal (SNP) baru pada intron telah ditemui pada kawasan 5`flanking ekson-3 di kalangan 46% daripada pesakit dan kontrol. Min penurunan tekanan mata adalah 33.1% pada bulan ke 6. Selepas 3 bulan rawatan dengan ubat titis mata latanoprost, sebanyak 47.4% pesakit glaukoma mencapai penurunan tekanan mata cecah 30% atau lebih (pesakit respons baik), 28.9% mencapai penurunan tekanan mata antara 15% hingga 30% (pesakit respons sederhana) dan 23.7% pasakit mencapai penurunan tekanan mata kurang daripada 15% (pesakit respons kurang). SNP Intron ini didapati tidak berkaitan dengan pesakit glaukoma, kaum dan keberkesanan ubat titis mata latanoprost.

Kesimpulan: Ubat titis mata latanoprost adalah berkesan di kalangan pesakit glaukoma tempatan. SNP intron pada gen PTGFR yang baru dijumpai ini tidak berkaitan dengan penyakit glaukoma dan keberkesanan ubat titis mata latanoprost.

ABSTRACT

Introduction: Diversity in clinical response among glaucomatous patients in response to topical latanoprost is not a new issue, many researchers attributing it to the different races, treatment regimes, glaucoma types, and some genetic variants.

Objective: The objective of this study is to determine the presence of polymorphism in exon of PTGFR gene and its role in responsiveness of glaucoma patients to topical latanoprost treatment.

Methodology: Glaucoma patients started on topical latanoprost were followed up for 6 months. Pattern of IOP and rate of good responder (30% reduction or more), moderate responder (15-30% reduction) and poor responder (less than 15% reduction) were determined. Polymorphism of the PTGFR protein coding region was identified among the glaucoma patients and the controls using denaturing High Performance Chromatography (dHPLC). The identified polymorphisms were associated with glaucoma and the pressure-lowering effect of topical latanoprost among glaucomatous patients on treatment.

Result: From 76 glaucoma patients and controls screened, no exon SNP was found. One novel intron $nSNP^{(A-T)}$ at 5`flanking region of exon-3 with frequency of 46% was identified. Among the glaucoma patients, mean IOP reduction was 33.1% and there was as high as 47.4% good responder, 28.9% of moderate responder and 23.7% of poor responder. The Intron SNP was statistically found to be neither associated with the responder rate, the race nor with glaucoma patients.

Conclusion: Topical latanoprost is effective among glaucoma patient in our local set up. The novel intron SNP found within the PTGFR gene does not statistically associate with glaucoma and clinical IOP lowering effect among glaucoma patients receiving topical latanoprost.

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Introduction

INTRODUCTION

1.1 BACKGROUND

Since the introduction of the latanoprost, the prototype of prostaglandin analogue in 1996, various clinical trials has concluded the effectiveness, side effects, possible complications, tolerability and also incidence of poor or non-responder.

Averages of 28 to 31 % of intraocular pressure (IOP) reduction by latanoprost monotherapy treatment were concluded in various clinical researches (Van der Valk et al, Zhang et al, 2005). The response to the latanoprost in various reviews was found to be less predictable and varies in subpopulations. For example in cases of non or poor responders to latanoprost, various studies had recorded figure at 10-20% but some studies recorded up to 50% of poor or non responder (Susanna and Medeiros, 2001; Hedman et al, 2002). A study comparing the effectiveness of the IOP reduction also found that Asian and Mexican glaucoma patients may be responding better to latanoprost over timolol than the corresponding population in the Caucasian (Hedman & Larsson , 2002).

Along with the reports of diversity in IOP lowering effects, it is observed that side effects were also varies in subpopulations; iris hyperpigmentation was only observed among Caucasian but hypertrichiosis, conjunctival hyperemia, was universal. Moreover, hyperchromic irises were found only in specific iris colored patients (Alm et al, 1994)

INTRODUCTION

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Along with the reports of diversity in IOP lowering effects, it is observed that side effects were also varies in subpopulations; iris hyperpigmentation was only observed among Caucasian but hypertrichiosis, conjunctival hyperemia, was universal. Moreover, hyperchromic irises were found only in specific iris colored patients (Alm et al, 1994)

Wadhwa & Higginbotham (2005) in a review raised an issue of whether these phenomenons can be explained by the distribution of the single nucleotide polymorphism (SNP) within the gene rather than ethnicity.

And thanks to recent advances in molecular genetic and genome sequencing techniques, pharmacogenetic researches in identifying genes and allelic variants of genes that affect our response to current drugs has gained enormous momentum (Wolf et al, 1997). With growing awareness of the pharmacogenetic studies and contemporary knowledge in efficient laboratory techniques, clinical observed response discrepancies found within populations or subpopulations were extensively under investigation with the vision of tailoring the therapies to individuals in the future.

Clinical trials on latanoprost published were mostly conducted in the western population but hardly any in our populations. Evidence and information regarding extend of effectiveness and validity of the labeled side effects of the drug is still vague in local set up. This dissertation was designed to study primarily the IOP lowering effects of latanoprost in the local glaucoma patients and its association with genetic polymorphism of the prostanoid (FP) receptor.

1.2 PHARMACOGENETIC

Pharmacogenetic refers to inborn variation in any group of creatures in response to xenobiotics (biologically active material formed outside host's body e.g. drug) (Kalow, 1997). Recently, pharmacogenetic research has gained tremendous momentum with the advances in molecular genetic and genome sequencing. The two main direction of pharmacogenetic studies were actually either identification of specific genes and gene products associated with various diseases (which may act as targets for new drugs) or identifying genes and allelic variants (such as SNP) of genes that affect our response to current drugs (Wolf et al, 2000).

Diversity of clinical response in various population and subpopulation had cited many researchers to correlate the phenomenon to racial differences. But with the fact that world population shares 99.9% of its genes and greater diversity in fact were found within the population instead of between them, study of polymorphism such as SNP distribution and its functional correlation would probably more relevant (Wadhawa & Higginbotham, 2005). Researchers were currently investigating genetic polymorphism that may be of therapeutical or toxicological importance with regards to clinical response of patients toward the prescribed medications.

1.3 RECEPTOR AND GENETIC POLYMORPHISMS

Polymorphisms were identified in more than 20 human drug metabolizing enzymes several with substantial race differences in their frequencies in year 2000. Some of them were critical determinants of therapeutic outcome (Wolf et al, 2000). One of the most studied enzymes would be the debrisoquine hydroxylase and its Cytochrome P450 coding CYP2D6 gene. Polymorphism of CYP2D6 gene is exhibited by the poor oxidation of debrisoquine (which is a sympatholytic antihypertensive agent) and is inherited as an autosomal recessive trait (CYP2D6 gene) in up to 10% of the British, German and Finnish population (Greenlee & Kwon, 2003). Cytochrome P450 CYP2D6 gene controls expression of the debrisoquine hydroxylase expression causes the diverse response to various psychiatric, cardiovascular and neurological drugs including beta-blocker such as timolol. Polymorphism of enzyme thiopurine methyltranferase which is responsible for the metabolism of the anti-tumour agents 6-mercaptopurine and 6 thioguanine is another example (Kalow, 1997).

Apart from enzymes polymorphism, receptor polymorphism and corresponding gene polymorphism is another identified potential determinant of therapeutic outcome that have been investigated in pharmacogenetic studies. For example, the β-receptor polymorphisms as consequences of mutagenesis had proven to alter the adrenergic receptor function. Typically the genetic polymorphisms influence the receptor expression, agonist and antagonist–binding affinities, physical and functional coupling to stimulatory G protein (G_S), receptor trafficking and receptor regulation by agonist (Liggett et al, 2000) (Table 1.1). Most of these receptor polymorphisms were resultants of the single nucleotide polymorphism (SNP).

Table 1.1: β-Receptor polymorphism and the phenotyping

Nucleic acid	Amino acid no	Designation	Phenotype
46	16	Arg-16	Reference
		Gly-16	Enhanced downregulation
79	27	Gln-27	Reference
		Glu-27	Absence downregulation
100	34	Val-34	Reference
		Met-34	Normal
491	164	Thr-164	Reference
		Ile-164	Decrease coupling, binding & sequestration
-47	19	5'LC Arg	Reference
		5'LC Cys	Increased expression

1.4 SINGLE NUCLEOTIDE POLYMORPHISM (SNP)

SNP were Deoxyribonucleic acid (DNA) sequence variation, occurring when a single nucleotide containing base such as adenine (A), thymine (T), cytosine (C) or guanine (G) in the genome is altered to another base. A variation must occur in at least 1-2% of the population to be considered polymorphism. In the late 90, there was an increase in interest towards SNPs among molecular geneticists because they were believed to be associated with the commoner complex familial disorders like diabetes, osteoporosis, cardiovascular or cancerous diseases. Potential merit of SNP as a marker for various diseases became the mission in many genome projects (Gray et al, 2000). Parallel to it, there is also growing of enthusiasm in association of genetic of drug response relating to SNP (Pharmacogenetic study).

SNP was estimated to cover the human genome at an average spacing of 1 SNP per 2000bp. In 2002, Haga et al (2002) resequenced 24 Japanese individuals and from 5% of the human genome analysed, they estimated frequency of SNP in the Japanese

population to be as frequent as one SNP in 807 bp . They have identified 174,269 SNP and 16,293 insertion/deletion polymorphisms within the screened gene region.

Although the presence of SNP can affect how humans handle diseases, bacteria, viruses, chemicals, drugs, etc., most of them do not. Miller & Kwok in their review on United State population had indicated that there are as many as ~17% of the candidates SNPs in the collection of databases had no detectable variation in any of the 3 major populations in the US (African-Americans, Asian, and Caucasians) and would not be useful for genetic studies.

About 6% of all SNPs are rare alleles which are always less than 20% in any population; ~53% are common SNPs that were 20% or more in any one population. Only ~27% of the SNPs candidates are common in all three populations (Miller and Kwok, 2001). Another aspect in pharmacogenetic is the study of Linkage disequilibrium (LD) among the potential SNPs. It is a measure of the degree of association between alleles in a population. The detection of disease-causing variants by association with neighboring single nucleotide polymorphisms (SNPs) depends on the existence of strong LD between them. LD could be disease linked or ethnic linked. Mukhopadhyay et al (2002) in a study on Indian patients discovered a total linkage disequilibrium of 2 SNP (- 83^{G-A} and 227^{G-A}) within the myocilin gene among the Asian population but weak linkage among the Caucasians.

In Ophthalmology, SNP found within the specific loci in the chromosome 1, 2, 3, 7, 8, 10, had been associated with risk of glaucoma. Apart from the well recognized

Myocilin (MYOC) and Optineurin gene, SNP on intervening sequence 8 of the OPA1 gene had been also recently found to be strongly associated with normal tension glaucoma but not higher pressure primary open angle glaucoma (Aung et al, 2001).

1.5 GLAUCOMA

1.5.1 DEFINITION AND PREVALENCE

Glaucoma represents a group of diseases with characteristic optic neuropathy and associated visual field loss for which elevated intraocular pressure (IOP) is one of the primary risk factors (AAO, section 10; 2000-2001). It is traditionally classified as primary (open or closed angle) glaucoma and secondary (open or closed angle) glaucoma. Primary open angle glaucoma (POAG) with normal or low intraocular pressure is known as normal tension glaucoma (NTG) or normal pressure glaucoma (NPG).

Glaucoma, especially POAG is a relatively low disability /case ratio disease which surface only in the past few decade as a major threat of blindness in Ophthalmology. Based on 111 published data, Harry Quigley estimated by 2000, the number of people with primary glaucoma to be at 66.8million and nearly 10% of them would suffer from bilateral blindness (Quigley, 1996). Even back in late 1980-early 1990, World Health Organization's (WHO) had estimated the incidence of primary open angle glaucoma (POAG) per year to be 2.4 million and the prevalence of POAG is between 1.1 to 2.1%

in general. It has been recognized as the second leading causes of blindness in the world now (Quigley, 1996).

Epidemiological studies in neighboring countries like Thailand found glaucoma as the second most common cause of unilateral blindness after cataract and in the study, it accounted for 11.2% of blindness (Bourne et al, 2003). In Singapore, Tanjong Pagar study found glaucoma was the largest contributory cause of blindness in population aged more than 40 year old (60%) followed by cataract and age related macular degeneration (Saw et al, 2004). Malaysian National Eye Survey, a population based study in 1996 estimated glaucoma to be merely the 5^{th} major cause of bilateral blindness (1.77%). But because the definition of glaucoma was far from satisfactory, the figure was generally agreed to be underestimated (Zainal et al, 2002).

Glaucoma patients generally present late especially the POAG and NTG. Late presentation had been shown to be the major contributory factors of blindness (29-41%) (Chen, 2004). Poor awareness of the disease would be another issue in the high prevalence of blindness in glaucoma. In a review, Hugh Taylor reported more than half of those in developing countries with glaucoma are actually unaware of the disease (Taylor & Keeffe, 2001) and even in developed countries, 75-78% of population had heard of glaucoma but only fewer than 10 % could actually identified a basic glaucoma definition correctly (Pfeiffer et al, 2002; Lau et al, 2002). In a recent cross-sectional study in our glaucoma population in Penang, Malaysia, Chieng et al found 8.1% of glaucoma patients were blind bilaterally and 14.9% had low vision at time of diagnosis. Almost one third of the diagnosed patients had demonstrated severe disc cupping of at

least 0.8 on presentation but 60.5% of them were still possessing satisfactory good vision of more than 6/18 or better at least in one eye (Chieng et al, 2005).

1.5.2 RACIAL DIFFERENCE IN GLAUCOMA

In general, the black race were not only found to have a higher prevalence of glaucoma, they also have younger presentation of the disease by 5-10 years, higher initial IOP, more severe course of disease and also postulated to have different efficacy of therapy due to heavier pigmentation of the eye (Wadhwa and Higginbotham, 2005; Grehn, 2001). They also scar more readily leading to poor success rate of glaucoma filtration surgery. Conversely, blacks were found to respond better to travaprost treatment by up to 2.4mmHg, although not with latanoprost and timolol treatment (Netland et al, 2001).

Hedman and Larson (2002) in a pooled 8 clinical trials analysis found Asian and Mexican patients showed a larger difference in mean diurnal IOP reduction between the timolol and latanoprost treatment as compare to patients from Europe and the United States. Greenlee and Kwan (2002) had emphasized the existence of different responses of treatment in various races and the necessity for clinicians to adjust the expectation of efficacy and side effect accordingly. Evidence was not strong enough to conclude the relationship between races and the drug responses in glaucoma management due to complex confounding factors such as socioeconomic and educational background.

Along with that, the differences that exist between races ultimately are still largely dictated by the genetic basis. Thus the genetic polymorphism would probably be the important factor rather than race in etiology of glaucoma and the response to drug

treatment in glaucoma patients. At least 2 trials had found the myocilin mutation incidence between the African American and the whites were almost equal (Wadhwa & Higginbotham, 2005) indicating the implication of genetic mutations in the aetiology of glaucoma.

1.5.3 MANAGEMENT OF GLAUCOMA

Glaucoma was once described by Fred Hollows as a disease which we do not know how to treat. It is a group of pathological entities with different pathophysiological mechanisms of action, characterized by ocular damage related in part to IOP. Recent researches have concluded pressure independent mechanism such as vascular and structural alteration of the optic nerve head may contribute in some cases to glaucomatous damage to the optic nerve (Tarek & Spaeth, *The Glaucomas-concept and fundamental*, 2000).

For centuries, the aim of management in glaucoma is mainly treating the obvious feature, which is the elevated IOP. Clinical evidence in effectiveness of treatment of glaucoma was vague (Wilensky, 1999) until Millennium when benefit of lowering IOP came to light, evidenced by various large clinical trials (Collaborative normal tension glaucoma study group, 1998; Heijl et al, 2002; The AGIS Investigators, 2000; The ocular hypertension treatment study, 2002). Current evidence does not find any lower limit of benefit of IOP lowering (Heijl et al, 2002; The AGIS Investigators, 2000).

The general principle in glaucoma management now is to maintain the patients' quality of life and quality of vision at a sustainable cost apart from control the IOP lower than target pressure. Target pressure is defined as the mean of an estimate IOP with treatment that is expected to prevent further glaucomatous damage (Tarek & Spaeth, *The Glaucomas-concept and fundamental*, 2000; European Glaucoma society, 2003). The initial target pressure is at least 30% lower than the initial IOP. This (30% reduction) has shown to reduce the rate of visual field progression from 35% to only 12% (Collaborative Normal Tension Glaucoma Study Group, 1998). The exact level of target IOP cannot be predicted with complete accuracy and IOP below 15 mmHg seen to be satisfactory in most but not all cases (Tarek & Spaeth, *The Glaucomas-concept and fundamental*, 2000).

Reducing IOP can be done via various options including topical or oral medications or engaging in surgical procedures like filtering surgery, trabeculotomy, or even laser therapy. Nevertheless, the main stay of treatment in POAG, NTG and OH currently is still the topical ocular hypotensive agents unless indicated (as in secondary glaucoma where relief of the primary cause would be the ultimate treatment or when topical anti-glaucoma is unlikely to provide effective IOP control).

Choices of ocular hypotensive agents were once limited, but now there are at least 5 different classes of anti-glaucoma agents to choose from which include beta blocker (e.g. timoptol, betaxolol etc), parasympathetic agonist (pilocarpine), carbonic anhydrase inhibitor (e.g. dorzolamide and brinzolamide), adrenergic agonists (e.g. propine, adrenaline) and alpha 2 agonist (brimonidine). Most are associated with major

side effects. Prostaglandin analogues like latanoprost, travaprost and bimatoprost are another newly discovered class of ocular hypotensive agents. They are certainly the most indispensable and inarguably the most effective ocular hypotensive agent available in the market now (Van Der Valk et al, 2005).

1.6 LATANOPROST

1.6.1 BACKGROUND

Since the introduction of latanoprost (first commercially available prostaglandin analogue) in 1996, prostaglandin analogues have become the most prescribed anti-glaucoma therapeutic agent. They were found to be the most effective ocular hypotensive agent in treatment of primary open angle glaucoma (Van der Valk et al, 2005). Other prostaglandin analogues available in the market are travaprost (Travatan 0.004%, Alcon) and Bimatoprost (Lumigan 0.03%, Allergan), which are both isopropyl ester and amide prodrugs respectively.

Topical Latanoprost 0.005% (Xalatan, Pfizer), is a synthetic prostanoid selective FP receptor agonist (isopropyl - (Z)-7 [(1R,2R,3R,5S) 3, 5 -dihydroxy- 2 - [(3R) – 3 – hydroxyl – 5 - phenylpentyl]cyclopentyl] – 5 - heptenoate with molecular formula of C_{26} H_{40} O_5). It is a prodrug with two long carbon chains (alpha and omega). The alpha chain is associated with easy penetration through the cornea where it is metabolized by an esterase enzyme in cornea to become latanoprost free acid (Ph XA 85). The omega

chain is responsible for the potency and selective FP receptor activation in the anterior chamber (Hylton & Robin, 2003).

It is well known that prostaglandin analogues reduce IOP via increased non-conventional uveoscleral outflow but the exact mechanism is still unknown. Researchers had found that in conjunction with the FP receptor binding, prostaglandin analogues interacts with the nucleus resulting in transcription of genes that biosynthesis pro-MMPs (matrix metalloprotease) which activates the MMPs (a group of collagenase important in remodeling of the collagen tissue). MMPs break down the collagen in the extracellular matrix and are being washed out with the aqueous as it flow through the uveoscleral pathway, thus opens up the pathway. Weinreb found exposure to prostaglandin $F_{2\alpha}$, 17-phenyltrinor-$PGF_{2\alpha}$, or latanoprost acid increased scleral permeability by up to 124%, 183%, or 213%, respectively; and MMPs were increased by up to 37%, 267%, and 96%, respectively. Transscleral absorption of FGF-2 was also increased by up to 126% with scleral exposure to latanoprost (Weinreb, 2001). This still does not explain the immediate IOP lowering effect seen within hours of administration (Hylton & Robin, 2003). Some researcher attributed it to the early relaxation of the ciliary muscle but is still unproven.

There is also evidence that latanoprost may influence cyclo-oxygenase and nitric oxide synthase activities and interfering with ischemia-induced neurotoxic processes and conferring neuroprotective postulation of the drug. In animal studies, significant increase in optic nerve head blood velocity has been found in rabbit and monkey eyes

after treatment with the latanoprost probably by relaxing the ciliary artery (Hylton & Robin, 2003).

1.6.2 INTRAOCULAR PRESSURE-LOWERING EFFECT

Latanoprost has been proven a well tolerable drug with effective IOP reduction of 28-31% (when used as Monotherapy) in POAG and Ocular hypertension patients, as shown in various control trial across various countries and races (Alm and Widengård, 2000; Liu et al, 1999; O'Donoghue et al, 2000; Aung et al, 2001; Hedman & Larsson, 2002; Tsukamoto et al, 2002; Kontas et al, 2003; Van der Valk et al, 2004; Thomas et al , 2005).

When topical latanoprost was used as adjunctive therapy to other class of ocular hypotensive agents, it was also found to be as effective. Addition of latanoprost (which enhances ourflow facility), to various aqueous suppressants such as timolol and carbonic anhydrase inhibitors, would logically produce a pure additive effect. Clinical trials have reported excellent additive effect of topical latanoprost as adjunctive therapy to various drugs with further IOP reduction of 23.2% to 26.1% irrespective of drugs used prior to the initiation of latanoprost (Bayer et al, 2002; Hoyng et al 1997).

With concern on the additive effect of latanoprost treatment in patients not controlled with topical timolol, the IOP reductions were found to be less consistent. Rulo et al (1994) reported that only a 13% further reduction was recorded at 2 weeks; conversely about 32% (4weeks) and 37% (12weeks) reduction was reported by Alm et al (1995). Hoyng et al (1997) in their study reported a large range of further reduction from 13%

to as high as 37%. Bron et al (2001) found further reduction of the IOP in the order of 25% by latanoprost at 2 and 6 week in patients adequately controlled with timolol alone or in combination with some other drug.

Recent clinical data also shows inconsistent findings with adjunctive latanoprost on timolol. It has been shown to produce an excellent further IOP reduction of 23.5% at 1month (Simmon et al, 2001) and 24.03% at 6 months (Diestelhorst et al, 2000); but Higginbotham et al (2002) reported only 10.65% reduction at 6 months of treatment. Nevertheless, should the baseline IOPs of the various studies be taken into consideration, we find the IOP reduction were rather consistent at 20-30%. With average baseline IOP of 20-25mmHg, IOP lowering effect among latanoprost adjunctive therapy was slightly lower than effect of reduction by monotherapy with magnitude of ~5%.

Pathophysiologically, the mechanism of action of beta-blockers are believed to via beta-adrenoceptor mediated modulation of the intracellular cyclic-Adenosine monophosphate ($_c$AMP) concentration in the ciliary epithelium, which affects the active transport capacity of cliliary epithelium, thus reducing aqueous production by as much as 50% (Hoyng et al, 1997). Adding of latanoprost to timolol would obviously induce pure additive effect although it does not proportionately shown, clinically.

Lately, small proportion of patients have been found to be non responsive to topical latanoprost and labeled as non responder. Although the definitions may differ slightly but generally non-responder rate of latanoprost in various subpopulation were estimated

from 10% to as high as 51.5%. Scherer (2002) found 25% of non responder (reduction less than 20% of baseline or less than 5mmHg reduction) in a retrospective review of 20 patients. Up to 13.5 % non-responders (less than 3mmHg at 20 hour after dose) had been documented by Netland et al (2001). In another study, 18-35% of latanoprost treated patients achieve less than 15% IOP reduction (Noecker et al, 2003). Choplin et al (2004) in 136 patients on latanoprost treatment found extremely high non responders rate ranging from 39.2%-51.5% (definition of <15% reduction). In Singapore, Aung et al (2001) have found only 11.3% to 14.8% of patients respond less than 15%.

Similarly, there has been different proportion with exceptionally good pressure lowering effects of responders (Camras and Hedman, 2003). Much needs to be answer as to what are the factors dictating this diversity of response. Attributing factors could well be racial difference, iris color difference, compliance or mere statistical error. Subpopulation genetic differences such as SNP are under research for various pharmacogenetic studies and we postulated that the diversity of response to latanoprost could be due to the diverse mutations within the population related to SNP.

1.6.3 SIDE EFFECTS

As with all other prostaglandin analogues, clinical efficacy of latanoprost comes at the expense of side effects. They are mainly conjunctival hyperemia 27.6% and foreign body sensation, prostaglandin induced iris pigmentation 5-15% (PIIP), hypertrichiosis 5.2%, possible breakdown of ocular blood barrier and cystoid macula oedema (Netland et al, 2001).

Clinical trials compared side effects among the prostaglandin analogues found the conjunctival hyperemia was least in latanoprost (Stewart et al, 2003). This is believed to be due to higher specificity of the drug to FP receptors and weaker agonist activity of latanoprost on the EP_2, IP and TP receptor which are known to mediate the vascular effects (Stjernschantz et al, 2000; Hoyng et al, 1997). Esterification of carboxylic acid group of the relatively hydrophilic latanoprost (prodrug) enhanced the corneal penetration and the activation of the prodrug by the esterase only occurs within the corneal. Apart from reducing the amount of drug required, it minimize the concentration of active compound at the surface, thus reduce the conjunctival hyperemia.(Hoyng et al, 1997).

PIIP and periocular skin pigmentation appears to be more prevalent in patients taking latanoprost (Hylton & Robin, 2003). However, none of the 60 Asian patients displayed PIIP after 2 months of exposure to latanoprost and unoprostone as reported by Aung et al (2001). Conversely, PIIP was a major concern in Caucasian, because of the lighter iris color in the population (Zhang et al, 2005).

There should be caution when prescribing latanoprost in high-risk patients with pseudophakic and aphakic eyes, as prostaglandin analogues were known to accelerate the disruption of the blood aqueous barrier and increase the incidence of cystoid macula oedema (Miyake & Nobuhiro, 1999).

Aung et al (2001) found that latanoprost is well tolerated in Asian patients with few ocular adverse events. About a third (32.43%) of their patients developed ocular irritation, 35% developed conjunctival redness, 2.7% developed skin rash and another 2.7% developed transient anterior chamber cells but none of the latanoprost patients stopped therapy due to side effects.

1.7 PROSTANOID (FP) RECEPTOR AND GENE

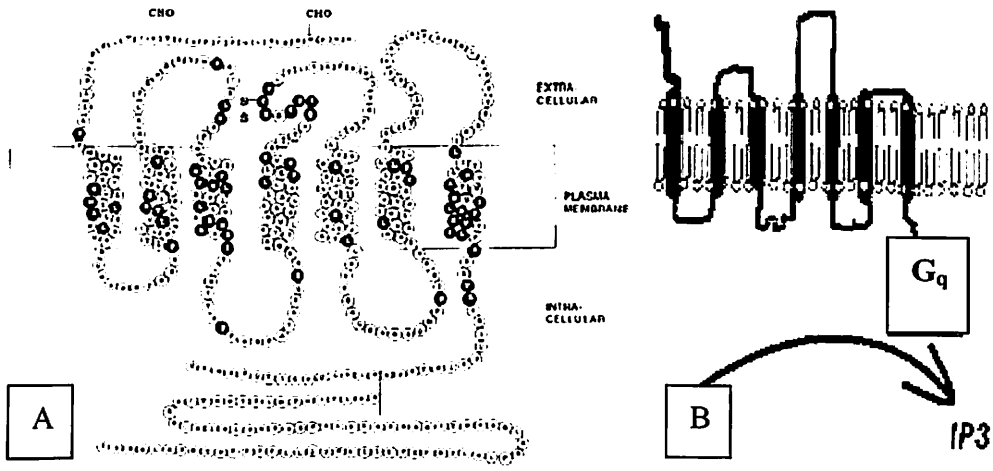

Figure 1.1: Showing the 7 putative transmembranous domains in the prostanoid receptor (A) and the G protein coupled activation of IP_3 (Inophosphate 3) (B) (Adapted from Narumiya et al, 1999)

1.7.1 PROSTANOID (FP) RECEPTOR:

Prostanoid receptors are the membrane receptors mediating the actions of the prostanoids. They are G protein-coupled receptors with 7 putative transmembraneous domains (Figure 1.1A). Prostanoids are locally acting autocrine or paracrine molecules with wide spectrum of biological activities. One receptor has been identified

pharmacologically for each natural occurring prostanoids depends on the individual affinity. Identified prostanoids include Prostaglandin (PG) D_2, $PGF_{2\alpha}$, prostacyclin (PGI_2) and thromboxane A_2 which has individual affinity towards their corresponding receptor named the DP-, FP-, IP- and TP receptors (-Rs). Four prostanoid receptor subtypes have been characterized pharmacologically for PGE_2, named EP1-R to EP4-R.

Selective agonist for virtually all prostanoids affect IOP, but among all PGF2α was identified in 1980s as the most effective ocular hypotensive agent. FP receptor inevitably became the receptor of interest among the researchers. Nevertheless, the underlying physiological mechanisms of IOP lowering are only partially understood.

The human FP receptor consists of 359 amino acid residues with a predicted molecular mass of 40,060. Activation of FP receptor leads to Gq mediated inositol phosphate 3 (IP_3) generation and subsequent increase in intracellular calcium (Figure 1.1B). FP receptor also coupled to other signal transduction systems (G-protein Rho mediated, phospholipid-C-β mediated etc).

Even though prostaglandins in general were well recognized as the important mediators of inflammation, there is little evidence to support FP receptors' role in inflammatory and immunological processes signaling (Hata & Breyer, 2004).

Expression of FP receptors is found in the corpus luteum of ovaries, myometrium which is linked to prolonged, painful menstrual bleeding and endometrial adenocarcinoma growth; in heart, they are associated with myocytes hypertrophy; in

stomach, FP receptor is linked to the protection of mucosa thus gastric ulcer; and in eye, it is associated with regulation of IOP. FP receptors are also found in the liver, respiratory tract and the kidney.

In the eye, only modest FP receptor is found in the ciliary muscle and virtually undetectable in NPE cells. Low level of mRNA was detected in corneal epithelium, ciliary processes, iris, inner retina, optic nerve and lens epithelial cells (Woodward et al, 1997). Introduction of $PGF_{2\alpha}$ and their analogs have been shown to lower intraocular pressure in humans which is believed to be mediated by the FP receptor.

Apart from that, melanocytes in eye were also found to express FP receptors. They stimulate expression and activity of tyrosinase thus promoting melanin synthesis. This mechanism is thought to be responsible for the side effects of PIIP (Scott et al, 2004).

1.7.2 PROSTANOID (FP) RECEPTOR GENE (PTGFR GENE)

In human, FP receptor is encoded by prostaglandin receptor $F_{2\alpha}$ gene (PTGFR), mapped to chromosome 1 (p31.1). It is a large gene consists of 3 exons spanning approximately 10kbp of genomic DNA separated by 2 large introns (Appendix A: human PTGFR gene). First exon is about 164b in size, second 870b and largest third exon is 1,459b in size. Intron 1 starts from position 165 (~1.3kb) and intron 2 starts from position 1035 (6.1kb) (Betz et al, 1999).

Exons literally represent the coding regions of a gene and are genetically priority of interest and introns conversely represent the non-coding region and biological significance of which are still opens to debate. Mutation within such a large gene is invariably present and inevitable. Distribution of various SNP in populations and subpopulation were known to varies, and to date 164 possible SNPs have been reported within the PTGFR gene in world populations. However, there were only 5 SNPs were reported within the 3 exons (Figure 1.2; Genbank database search dated Oct 2005).

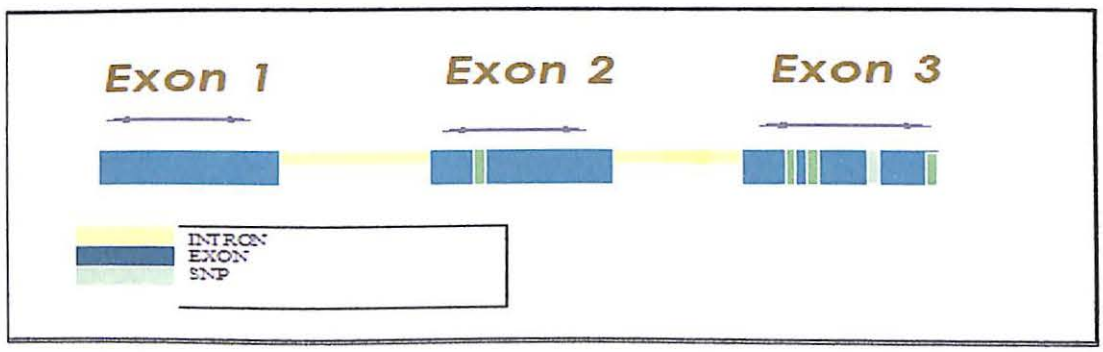

Figure 1.2: Schematic drawing representing the location of the existing Single nucleotide polymorphisms (SNP) found within the exon of the PTGFR gene (Schematic drawing based on Genbank database search dated Oct 2005)

Functional significance of these SNPs is still not known to mankind. The potential implications of these SNPs to the diseases, and drug response are still waiting to be explored. Oguma et al (2004) their research on PTGDR (prostaglandin D receptor gene) polymorphism have reported that various haplotype and diplotype of PTGDR sequence variants had large functional influence on the patients' susceptibility to asthma.

There has been extensive research in pharmacogenetic and polymorphism in the past few years but very few were done on PTGFR gene. Only research close to this gene were done by Sossey et al (2001) who reported loss of heterozygosity (LOH) of 1p31 chromosomal region in nearly 50% of human breast cancer patients. Presence of tumor suppressor gene within the gene was suggested.

The available data on prevalence of SNP was based on Caucasians thus do not represent our continent because ethnicity and racial origin in various continents displayed different SNP. Thus, exploration of the SNP of PTGFR gene within our population needs to start somewhere. It would serve as a corner stone for the relevant pharmacogenetic study not only in the field of Ophthalmology but in the other disciplines especially the Obstetric and Gynecology field.

1.8 ADVANCES IN GENETIC LABORATORY STUDIES

1.8.1 INTRODUCTION

Currently there has been revolutionary discovery of various laboratory techniques used in genetic studies which includes Polymerase Chain Reaction (PCR), Southern Blot analysis, Restricted Fragment Length Polymorphism (RFLP), Denaturing High Performance Liquid Chromatography (DHPLC) and etc. Selection of most efficient and cost effective method are necessary to avoid financial and man power wastage.

1.8.2 POLYMERASE CHAIN REACTION (PCR)

This technique was developed by Kary Mullis whom later received the Nobel Prize in chemistry in 1993. It is an efficient technique allows amplification of a selected DNA sequence in a genome a millionfold or more, provided the nucleotide sequence of at least one short DNA segment on each side of the region of interest is known.

PCR involve 3 steps:

1. Step one: Genomic DNA containing the sequence to be amplified is denatured by heating.
2. Step two: The denatured DNA is annealed to an excess of the synthetic oligonucleotide primers.
3. Step three: DNA polymerase (taq polymerase) is used to replicate the DNA segment between the sites complementary to the oligonucleotide primers.

The primer provides the free 3'-OH required for covalent extension and the denatured genomic DNA provides the required template function. The product from one cycle would then undergo second cycle which will eventually produce an exponential amplification.

Products of PCR can be detected and confirmed by gel electrophoresis. PCR techniques are the prerequisite for product purification in most of the SNP detection techniques including dHPLC.

1.8.3 DENATURING HIGH PERFORMANCE LIPID CHROMATOGRAPHY (dHPLC)

There are multiple contemporary methods used for the detection of unknown mutations which include single-strand conformation polymorphism analysis (SSCP), denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), mismatch cleavage methods (MCM), direct sequencing, oligonucleotide arrays, as well as gel electrophoresis and most importantly high-performance liquid chromatography-based heteroduplex detection (Huber et al, 2000).

DHPLC identifies mutations and polymorphisms based on observation that heteroduplexes will, under partially denaturing conditions, be more likely to denature, compared to their homoduplex counterparts (Meldrum et al, 2003). Under partially denaturing temperatures, the heteroduplexes elute from the column earlier than the homoduplexes because of their reduced melting temperature. DHPLC is a recently developed technique popularized by P.J. Oefner. Advantages of dHPLC include high sensitivity and specificity, flexibility of fragment size, low labor intensity and operator time (O'Donovan et al, 1998). It has emerged the most sensitive method for high throughput polymorphisms detection (O'Donovan et al 1998; Sivakumaran et al., 2003; Meldrum et al, 2003).

O'Donovan et al (1998) has reported 100% sensitivity and specificity for fragments less than 1000bp. Sivakumaran in his review (2003) had found as high as 96% (upto 670bp, Jone's et al), 97% (Escary et al), 99.44% (Wagner et al) and 100% (Gross et al) sensitivity in 4 studies. The major limitations in various techniques detecting single