ASSOCIATION OF GENETIC MARKERS OF *PLEKHA7*, *ABCC5* AND *KALRN* AND PROGRESSION OF PRIMARY ANGLE CLOSURE GLAUCOMA IN MALAY PATIENTS

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DISCLAIMER

I hereby certify that the work in this dissertation is my own except for the quotations and summaries which have been duly acknowledged.

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ABSTRAK

Glaukoma merupakan antara penyakit mata yang menjadi penyebab utama kebutaan, dengan separuh daripada kes glaukoma di seluruh dunia terdiri daripada masyarakat Asia. Glaukoma primer sudut tertutup (GPST) merupakan penyakit yang lazim di dapati di kalangan penduduk Asia. Terdapat variasi dari segi prevalens GPST mengikut bangsa di Asia. Bangsa Melayu dikenalpasti menghidapi GPST dengan prevalens sebanyak 0.12% hingga 2.5%. GPST pada masyarakat Asia didapati lebih agresif dan progres dengan kadar yang cepat berbanding masyarakat Eropah. Ini menyebabkan kadar kebutaan yang tinggi dalam kalangan penduduk Asia. Mengenalpasti penanda genetik yang berkecenderungan kepada progres penyakit GPST adalah penting untuk mencegah kebutaan dan penemuan baru rawatan pada masa akan datang.

OBJEKTIF

Kajian ini bertujuan untuk mengenalpasti perkaitan di antara tiga penanda genetik iaitu *PLEKHA7, ABCC5* dan *KALRN* dengan progres penyakit GPST di kalangan pesakit Melayu.

METODOLOGI

Kajian rentas melibatkan 163 orang pesakit GPST dari Hospital Universiti Sains Malaysia dan Hospital Raja Perempuan Zainab II telah dijalankan di antara April 2015 hingga April 2017. Darah vena sebanyak 6 ml diambil dari semua pesakit. Pengekstrakan DNA telah dijalankan menggunakan kit pengekstrakan DNA komersil

(QIAGEN, Germany). Pengoptimuman primer dijalankan ke atas rs11024102 gen PLEKHA7, rs17217796 gen *ABCC5* dan rs1392912 gen *KALRN*. Reaksi berantai polimerase (PCR) dijalankan dan diikuti oleh pembersihan amplikon. Polimorphisme nukleotida tunggal (SNP) dikenalpasti melalui proses penjujukan DNA. Medan ujian penglihatan menggunakan Humphrey Visual Field (HVF) dijalankan semasa calon pesakit di pilih. Perubahan medan penglihatan dibandingkan dengan hasil ujian semasa pertama kali diagnosa dibuat. Berdasarkan persetujuan diantara dua sistem skor ke atas ujian medan penglihtan; Advanced Glaucoma Intervention Study (AGIS) dan Hodapp-Parrish- Anderson (HPA), pesakit dibahagikan kepada kumpulan yang progres dan tidak progres. Ujian statistik 'Chi-Square' digunakan untuk menganalisa perkaitan di antara penanda aras genetik dan progres penyakit GPST.

KEPUTUSAN

163 orang pesakit berbangsa Melayu dengan GPST direkrut dalam kajian ini (58 orang lelaki dan 105 orang perempuan). Keputusan kajian mendapati bahawa 29 (18%) orang pesakit didapati menunjuk penyakit yang progresif setelah di rawat selama 6 tahun (SD 1.0) tahun. Kekerapan allele minor (MAF) untuk *PLEKHA7* rs11024102 (G/A), *ABCC5* rs17217796 (C/G) dan *KALRN* rs1392912 (A/G) adalah 0.44, 0.08 and 0.48 masing-masing. Tiada pebezaan yang signifikan terhadap kekerapan alele rs11024102 (p=0.828), rs17217796 (p=0.865) dan rs1392912 (p=0.684) diantara pesakit GPST yang progres dan tidak progres.

KESIMPULAN

Kajian ini mendapati walaupun penanda aras genetik *PLEKHA7* dan *ABCC5* mempunyai perkaitan dengan risiko GPST, namun tidak mempengaruhi progres GPST pada pesakit berbangsa Melayu. Kajian ini juga menunjukkan KALRN gene tidak mempengaruhi progres GPST. Berkemungkinan terdapat penanda aras genetik yang lain yang lebih cenderung kepada progres GPST.

ABSTRACT

INTRODUCTION

Glaucoma is the leading cause of irreversible blindness worldwide, with Asians accounting for approximately half of the world's glaucoma cases. Primary angle closure glaucoma (PACG) is highly prevalence among Asians. The prevalence of PACG varies widely according to the different races in Asia. PACG prevalence in Malays ranges from 0.12% to 2.5%. Asians patients with PACG are found to progress faster than Caucasians. PACG is responsible for more blindness in Asian population. Identifying the potential susceptible genetic markers for progression of PACG in Malays is essential for management strategy to prevent blindness and future development of new treatment.

OBJECTIVE

To determine association of genetic markers of *PLEKHA7*, *ABCC5* and *KALRN* and progression in PACG Malay patients.

METHODOLOGY

A cross sectional study was conducted between April 2015 and April 2017 involving Malay patients with PACG from Hospital Universiti Sains Malaysia and Hospital Raja Perempuan Zainab II (Kota Bharu). Venesection was performed. DNA extraction was conducted using commercialize DNA extraction kit (QIAGEN, Germany). Optimization of primer was conducted for rs11024102 of *PLEKHA7*, rs17217796 of *ABCC5* and rs1392912 of *KALRN*. Polymerase chain reaction (PCR) was carried out

using Thermocycler SureCycler 8800 (Agilent Technologies, Santa Clara, CA). PCR products were purified using Illustra Exostar (Ge Healthcare Bio-Science) PCR product purification kit. PCR products were sent to a private laboratory (First Base laboratories, Selangor, Malaysia) for cycle sequencing. Humphrey visual fields (HVF) were conducted during study recruitment. HVF obtained were compared with two baseline visual field. Patients were grouped into progress and non-progress based on the agreement of both AGIS and Hodapp-Parrish scoring system on HVF. Chi Square test was used to analyse association of genetic markers and progression of PACG.

RESULTS

A total of 163 Malay patients were recruited with PACG primary (58 men and 105 women). Forty nine or approximately 30% of patients had acute primary angle closure attack. There were 29 (18%) patients with visual field progression of PACG after a mean follow up of 6.0 (SD 1.0) years. Minor allele frequency (MAF) for *PLEKHA7* rs11024102 (G/A), *ABCC5* rs17217796 (C/G) and *KALRN* rs1392912 (A/G) is 0.44, 0.08 and 0.48 respectively. There was no statistically significant association between rs11024102 (p=0.828), rs17217796 (p=0.865) and rs1392912 (p=0.684) with progression of PACG in Malay patients.

CONCLUSION

Although genetic markers of *PLEKHA7* and *ABCC5* were found to associate with the risk of PACG but they have no role on progression of PACG in Malay population. In this study, KALRN gene did not show any role on progression of PACG in Malay population. Perhaps, there are other susceptible genetic markers responsible for progression in PACG.

Chapter 1
Introduction

1.1 GLAUCOMA

Glaucoma is a disease that has been described in literature as early as 400BC, where the term "glaukos" means blue, green and cloudy. British oculist Richard Banister described it as the firmness of the eye, however in the 18th and 19th century it was thought to be a disease of the vitreous humour, arthritis and iritis. This was disapproved by Helmholtz in 1851 who noted that the vitreous was normal but the optic nerve was cupped. Curran put forth the pupillary block theory in 1920s (Curran, 1920). Not long after this, the gonioscope was invented by Trantas, Salzmann and Koeppe (Consoli *et al.*, 2005).

Glaucoma is a group of chronic progressive optic neuropathies comprising of a slow progressive degeneration of the retinal ganglion cells and their axons, resulting in structural changes of the optic disc and a corresponding pattern of visual field loss (Weinreb and Khaw, 2004). It is a complicated disease with many underlying mechanisms and a complex relationship with intraocular pressure (IOP). In 2000, The Advanced Glaucoma Intervention Study showed the importance of IOP in glaucoma progression (Investigators, 2000). This study found that patients with IOPs of <18mmHg (average, 12.3mmHg) were unlikely to have visual field progression. This study confirmed that having good IOP control will slow disease progression.

Glaucoma can be classified into two main groups according to the angle structure; closed angle glaucoma and open angle glaucoma (Coleman, 1999). Open and closed angle glaucoma can further be classified into primary or secondary glaucoma. Primary glaucoma of open angle category includes primary open angle glaucoma (POAG), juvenile open angle glaucoma (JOAG), normal tension glaucoma (NTG) and congenital glaucoma (Foster, 2002). The most common type may differ from one

region of the world to another. For instance, primary angle closure glaucoma (PACG) is more prevalent in certain regions in Asia, whereas POAG is more equally distributed throughout the world and is the most common form of the disease, accounting for 74% of all glaucoma cases (Quigley & Broman, 2006).

1.2 PRIMARY ANGLE-CLOSURE GLAUCOMA

Acute angle closure glaucoma is diagnosed when there is an occurrence of a congestive episode with headache, blurring of vision, halos around the light with associated eye redness and pain and the presence of markedly raised IOP in an eye with shallow anterior chamber, vertically oval pupil, corneal edema and gonioscopically closed angle (Foster *et al.*, 2002). Chronic angle closure glaucoma (CACG) represents chronically elevated IOP, in association with an angle in which at least 180° of trabecular meshwork was not visible on gonioscopically, confirmed peripheral anterior synechiae which is irreversible, optic nerve head and visual field changes with or without prior symptoms (Foster and Johnson, 2000). In a study done in India, Sihota *et al* (2011) reported that 80% of primary angle closure glaucoma is asymptomatic. Many studies have shown similar observation, thus it was coined "sneak thief" due to almost asymptomatic course until the very advanced stage of the disease (Ichhpujani *et al.*, 2010; Liza-Sharmini *et al.*, 2014).

1.2.1 Global prevalence of PACG

Previously, the estimated global mean prevalence for OAG in 2010 was 1.96% while ACG was 0.69% (Quigley & Broman, 2006). However, based on the results obtained from latest systemic review and meta-analysis, showed that the overall prevalence of glaucoma for the population aged 40 to 80 years was 3.54% of which 3.05% was

attributed by POAG and 0.50% by PACG in 2013 (Tham *et al.*, 2014). The analysis also stated that the number of people (aged 40-80 years) with glaucoma may increase from 64.3 million (2013) to 76.0 million in 2020 and 111.8 million in 2040. The prevalence of PACG varied across geographic regions with the highest prevalence of PACG being Asia (1.09%; 95% CI, 0.43-2.32) (Tham *et al*, 2014). The prevalence of POAG has always been higher than PACG in the western world (Klein *et al.*, 1992; Mitchell *et al.*, 1996).

However, in 2012, European based study concluded that PACG is more common than previously thought. Their result showed 130 000 people in the UK, 1.60 million people in Europe and 581 000 people in the USA with PACG in 2012. They predicted cases will increase by 19% in UK, 9% in Europe and 18% in USA within the next decade, on account of ageing (Day *et al.*, 2012). Asia constitutes for a disproportionately higher prevalence of PACG as opposed to the prevalence of POAG cases which are more evenly distributed throughout the world (Quigley, 1996). In 2010, higher prevalence of PACG cases were seen in Asian countries; China 1.26%, Southeast Asia 1.20%, India 0.80%, as compared to the lower prevalence seen in other parts of the world; Europe 0.25%, Latin America 0.19%, Africa 0.16%. Japan and the Middle East registered lower than average prevalence of PACG; 0.39% and 0.16% respectively (Quigley & Broman, 2006). Therefore, Asians represent 87% of the 15.7 million with ACG (Quigley & Broman, 2006).

1.2.2 Prevalence of PACG in South East Asia

There are variations in glaucoma prevalence across ethnicity and Asians had the highest prevalence of PACG (1.20%; 95% CI, 0.46-2.55) than it is in Africans or

Europeans and is a significant cause of visual morbidity in East Asia (Congdon *et al.*, 1992; Foster *et al.*, 1996; Foster *et al.*, 2000).

Studies conducted in southern states of India have shown a varied prevalence of PACG between 0.5% to 4.3% (Dandona *et al.*, 2000; Ramakrishnan *et al.*, 2003; Vijaya *et al.*, 2006). Various epidemiologic population-based studies in East Asia and Southeast Asia shows variation in the prevalence according to different parts of Asia; Chinese 1.3% (He *et al.*, 2006), Mongol 1.4%(Foster *et al.*, 1996), Thai 0.9% (Bourne *et al.*, 2003). The Meiktila Eye study in rural Myanmar showed the prevalence of glaucoma in the population aged ≥40 years in rural, central Myanmar was 4.9%. The ratio of PACG to POAG was approximately 1.25:1 (Casson *et al.*, 2007). Malays are among affected races in South East Asian with PACG prevalence of 0.12% to 2.5% (Bourne, 2003; Casson *et al.*, 2007; Shen *et al.*, 2008).

1.3 GLAUCOMA PROGRESSION AND SEVERITY

Glaucoma is a chronic progressive disease. Traditionally, the diagnosis of glaucoma was based on three criteria: an increased in IOP, typical visual field defects, and characteristic optic disc damage. However, IOP value alone can neither be used to distinguish healthy from affected individuals nor to stage the disease according to its severity, due to its poor sensitivity and specificity (Brusini and Johnson, 2007). The disease causes damages to ganglion cell and its respective axons as well, resulting in progressive and asymmetric changes in the optic cup, with corresponding visual field loss. Often visual field changes are preceded by structural changes, about 40% retinal nerve fibre death occurs before detectable visual field changes (Tuulonen and Airaksinen, 1991). Therefore, glaucomatous damage can be quantified using either

structural (changes in the optic nerve and retinal nerve fibre layer) or functional loss (visual field defects), or a combination of both (Brusini and Johnson, 2007; Medeiros *et al.*, 2012). The rate of progression varies highly among patients (Leske, 2007; Rossetti *et al.*, 2010). Disease progression in glaucoma is common and despite treatment, the majority of patients still progress (Rossetti *et al.*, 2010).

1.3.1 Evaluation of visual field progression

Detection of progression plays an important role in the clinical diagnosis and management of glaucoma. Standard automated perimetry (SAP) remains the method of choice for monitoring functional changes in the disease. Various studies have chosen visual field outcome as an end point (Schulzer, 1994; Katz *et al.*, 1999; Keltner *et al.*, 2000; Heijl *et al.*, 2008). The evaluation of visual field progression can be achieved by employing trend analysis and event-based analysis (Birch *et al*, 1995; Heijl *et al*, 2002; Spry and Johnson, 2002; Diaz-Aleman *et al*, 2009).

Table 1.3 Examples of event and trend base analysis

Event based analysis	Trend based analysis
Advanced glaucoma intervention study criteria	Linear regression analysis
Collaborative initial glaucoma treatment study	Guided progression analysis
Hodapp-Parish-Anderson criteria	Statpac
Mill's staging system	Statpac 2
Early manifest glaucoma treatment study criteria	Progressor
Glaucoma progression analysis 1	
Glaucoma progression analysis 2	

1.3.1.1 Trend base analysis

Trend analysis uses mean deviation index (MDI) or visual field index (VFI) calculated from the Humphrey visual field (HVF) perimetry. It has become a standard index for estimating the progression rate of glaucoma (Casas-Llera *et al.*, 2009). Nonetheless, MDI calculation can be influenced by the presence of cataract correlate poorly with clinical findings. Thus, progressive increase in cataract density can falsely be mistaken as high glaucoma progression rate (Heijl *et al*, 1986; Klein *et al*, 1996; Koucheki *et al*, 2004). The value of MDI will improve after cataract extraction and this may further interfere with the evaluation and monitoring of glaucoma progression (Klein *et al*, 1996, Koucheki *et al*, 2004). Another limitation of using MDI is that it is very weakly centre weighted, therefore it does not correlate well to patient's real visual function (Heijl *et al*, 1986).

1.3.1.2 Event-based Analysis

The event-based analysis is essential to detect whether progression has occurred or not (Caprioli, 2008). The are many examples of standard automated perimetry methods to detect progression (Hodapp *et al.*, 1993). AGIS investigators developed scoring system; 0 (no defect) to 20 (all test sites deeply depressed). Visual field considered progress if there's a worsening of 4 units in the AGIS score sustained during 3 consecutive 6-month visits (Investigators, 1994).

Mills and colleagues had proposed a staging system by upgrading Hodapp-Parish-Anderson criteria (Mills *et al.*, 2006). There are three factors on categorising of stages; stages 0 and 1 depends on pattern standard deviation (PSD) and hemifield test results,

stages 2 through 4 are adjusted by numeric (dB) plot, and the pattern deviation plot is used for 1 through 4. Stage 5 classifications are on the basis of poor visual acuity and inability to perform visual field testing as a result of a severe loss of vision. Progression was considered when visual field progress from one stage to the next (Mills *et al.*, 2006).

Glaucoma progression analysis (GPA) software incorporated in Humphrey Visual Field Analyser (HVA) (Carl-Zeiss Meditec, Dublin, CA) is an example of event-based analysis (Casas-Llera *et al.*, 2009). The software will give an analysis of pattern standard deviation values (Casas-Llera *et al.*, 2009) allowing for glaucoma progression monitoring. Recently, glaucoma progression index (GPI) was introduced to measure the rate of VF progression (Bengtsson *et al.*, 2009). It was found that GPI analysis is more accurate than the traditional MDI analysis for determining the rate of progression and is considerably less affected by cataract or cataract surgery (Bengtsson and Heijl, 2008).

The event-based GPA analysis is capable of detecting progression earlier compared to trend VFI analysis by 7 months (Casas-Llera *et al.*, 2009). Trend-based analysis requires a larger number of HVF test to detect progression (Caprioli, 2008). An innovative study combining both event and trend based analysis by building a hierarchical Bayesian model was done by Medeiros and colleagues in which it shows promising reliability in measuring visual field progression outcome (Casas-Llera *et al.*, 2009).

1.3.2 Evaluation of structural progression

The progression of glaucoma can also be monitored by the structural changes of the optic nerve head (ONH). According to these studies; collaborative initial glaucoma treatment study (CIGTS) and ocular hypertension treatment study (OHTS), structural damage may be a more sensitive indicator of glaucomatous progression than functional damage in patients with glaucoma or ocular hypertension (OHT) (Kass *et al.*, 2002). With the advancement of technology, newer and more sophisticated ophthalmic imaging devices have been introduced such as Heidelberg retinal tomograph (HRT) and optical coherence tomograph (OCT). These non-invasive imaging tools provide us with quantitative images and allow for precise observation, documentation and monitoring of the optic nerve head , retinal nerve fibre layer and inner macular layer (Medeiros *et al.*, 2009; Bussel *et al.*, 2013). However, some authors recommended determining progression on the agreement and correspondence between structural progression and functional deterioration (Leung *et al.*, 2010; Medeiros *et al.*, 2011).

1.3.3 Staging and severity

1.3.3.1 Advanced Glaucoma Intervention Study (AGIS)

A continuous glaucoma staging systems have been recommended by the Advanced Glaucoma Intervention Study. In this scoring system, the severity of glaucoma can be quantified using the Humphrey 24-2 threshold test. The AGIS visual field defect score is based on the number and depth of clusters of adjacent depressed test sites in the upper hemifield, lower hemifield and in the nasal area of the total deviation plot (an event-based analysis) (Investigators AGIS, 1994; Ng *et al*, 2012). The scores for each hemifield and nasal area are summed up and visual field scores are divided into five

categories: 0 = normal visual field; 1-5 = mild damage; 6-11 = moderate damage; 12-17 = severe damage; and 18-20 = end stage (Investigators, 1994). This staging system is almost accurate and provides standardised classification of visual field according to severity. Thus, it is very useful for scientific and clinical research. However, it is time-consuming, requires special training and not practical for day-to-day clinical usage (Brusini and Johnson, 2007).

1.3.3.2 Hodapp-Parish-Anderson (HPA)

HPA classification system considers two criteria: the first criterion is the overall extent of damage using both the mean deviation (MD) value and the number of defective points in the Humphrey Statpac-2 pattern deviation probability map of the 24-2, SITA-STANDARD test; the second is based on the defect(s) proximity to the fixation point (Hodapp *et al.*, 1993; Susanna Jr and Vessani, 2009). This classification, though popular has its own disadvantages, namely the visual field defect is characterised into four relatively course stages and does not give information about the location and depth of the defect(s). It is time-consuming and impractical for day to day analysis of every VF test results. Another limitation is this system may suggest a significant deterioration when in fact none has occurred (Susanna Jr and Vessani, 2009).

1.3.4 Factors affecting visual field progression

Age as a risk factor for glaucoma progression has been confirmed by many clinical trials (Gordon *et al.*, 2002; Leske *et al.*, 2003; Musch *et al.*, 2009). Not all patients with elevated IOP is considered to have glaucoma (Linner & Stromberg, 1964; Armaly MF, 1969; Spaeth GL, 1994) and around one-third or more of those with glaucomatous

optic discs changes and visual field (VF) defects did not have elevated IOP (Hollows & Graham, 1966; Shiose Y, 1983; Spaeth GL, 1994). Once diagnosed as glaucoma, elevated IOP during follow up was a strong factor for glaucoma progression with the hazard ratio increasing by 11% for every 1 mmHg of higher IOP in Early Manifest Glaucoma Trial study (EMGT) (Heijl *et al.*, 2002). Large IOP fluctuations may be associated with disease progression in PACG eyes (Gazzard *et al.*, 2003; Tan *et al.*, 2015).

In a 5 year observational study done in India, it showed that the progression rate and mean IOP was higher in patients who did not receive laser peripheral iridotomy (Thomas *et al.*, 2003). While other similar study done elsewhere predicted that laser peripheral iridotomy can prevent progression as it can contribute to lower/controlled IOP at an initial stage (Nolan *et al.*, 2000). Some studies predicted laser peripheral iridotomy is not enough, chronic angle closure glaucoma often requires further surgical interventions (Alsagoff *et al.*, 2000; Nolan *et al.*, 2000).

Female gender, migraine and disc haemorrhage have also reported to be the risk factors associated with progression in normal tension glaucoma (NTG) (Drance *et al.*, 2001; Skaat *et al.*, 2016). In EMGT study, disc haemorrhage and thinner central cornea thickness (CCT) are also significant glaucoma progression factors (Leske *et al.*, 2007). On the other hand, longer follow up and a higher number of glaucoma interventions are associated with visual field progression in AGIS study (Nouri-Mahdavi *et al.*, 2004)

1.4 GENETICS

Advances in glaucoma genetics have increased our knowledge on genetic factors contributing to the development of most types of glaucoma. Study of genetics has evolved ever since Human Genome Project was completed in 2003 and the International HapMap Project in 2005. This became a platform for researches to look into genetic contributions to common diseases. The project is a research tool with computerised databases containing reference human genome sequence and a map of human genetic variation that allow any researchers to analyze whole-genome samples for genetic variations that contribute to the onset of a disease (Bush and Moore, 2012). A major outcome of the project is through genome wide association study (GWAS),. This method searches the genome for small variations, called single nucleotide polymorphisms or SNPs (pronounced "snips"), that occur more frequently in people with a particular disease than in people without the disease (Hirschhorn and Daly, 2005). Each study can look at hundreds or thousands of SNPs at the same time. Researchers use data from this type of study to pinpoint genes that may contribute to a person's risk of developing a certain disease. An archive of data from genome-wide association studies on a variety of diseases and conditions already can be accessed through an NCBI Web site, called the Database of Genotype and Phenotype (dbGaP) located at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gap.

1.4.1 Genetics in glaucoma

Mostly, patients exhibit autosomal dominant or autosomal recessive inheritance, for early onset disease (less than age 35). Whereas adult-onset glaucoma has complex inheritance pattern due to multiple genetic and/or environmental risk factors influences. Some of the discoveries made; for example, two genes *CYP1B1* coding for cytochrome P450 1B1 and *LTBP2* has been discovered to cause congenital glaucoma (Lim *et al.*, 2013). Axenfeld-Rieger syndrome, aniridia, and glaucoma associated with anterior segment dysgenesis are caused by mutations in *PITX2*, *PAX6*, and *FOXC1*, respectively (Lee *et al.*, 2008; Reis *et al.*, 2012). To date, POAG has been linked to 20 genetic loci but only 3 causative genes were identified from these loci; myocilin (*MYOC*), optineurin (*OPTN*) and WD repeat domain 36 (*WDR36*) (Fan *et al.*, 2006). Whereas PACG related studies conducted within Asian populations identified significant polymorphisms with the *PLEKHA7* and *COL11A1* genes and an intergenic region between *PCMTD1* and *ST18* on chromosome 8q (Vithana *et al.*, 2012). *KALRN* gene was identified as susceptible genetic marker for progression of POAG in Malay patients. KALRN gene plays important role in Rho associated protein kinase (ROCK) pathway. ROCK pathway is involved in aqueous humor drainage, improvement in retinal circulation and potential neuroprotective effect (Inoue and Tanihara, 2013; Wang and Chang, 2014). Perhaps, potentially KALRN gene may also affect the risk of progression in PACG. To date, no study conducted involving *KALRN* gene and PACG.

1.4.2 Genetics in PACG

A gene is a sequence of deoxyribonucleic acid (DNA) that carries the information representing a protein (Lewin, 1987). The building blocks of DNA are nucleotides that are arranged in pairs and form a double helix. The 23 pairs of chromosomes that embody the human genome are comprised of approximately 3.12 billion sequence nucleotide pairs. These nucleotides are arranged in highly conserved sequence. It is estimated that the sequence differences between any human are less than 1%.

The most common sequence difference observed in humans is called single nucleotide polymorphism (SNP). This is a sequence change that alters a single nucleotide pair in a sequence of DNA, which can code for a change in structure or expression of a protein. These change can be beneficial or have a negative effect. Thus polymorphism is any genetic variation that is present within a population that occurs with a relatively high frequency, usually considered more than 1% (Karki *et al.*, 2015). Most polymorphism does not cause an identifiable change in the organism in which it occurs. However, it may alter disease susceptibility and drug response.

The earliest PACG study was done by Tornquist (1953). He suggested that angle closure glaucoma was transmitted by a single dominant gene. Unfortunately, studies could not prove the hypothesis (Tornquist, 1953). However, the genetic link to PACG is more of associated with single nucleotide polymorphism (SNP). The relationship between SNPs of the extracellular matrix, matrix metalloproteases (MMP) and PACG was studied by Jang and colleagues. They concluded that SNP of the MMP gene is likely to be associated with acute PACG (Wang *et al.*, 2006). More studies have been

conducted to understand the molecular basis of this major cause of blindness. Recent discoveries of SNPs which were significantly related to PACG were *PLEKHA7*, *ABCC5* and *COL11A1* (Khor *et al.*, 2016; Nongpiur *et al.*, 2014; Vithana *et al.*, 2012). So far, only POAG related genetic study manage to show strong association with disease progression (Trikha *et al.*, 2015).

1.4.2.1 *PLEKHA7*

PLEKHA 7 (Pleckstrin homology domain-containing family A member 7)

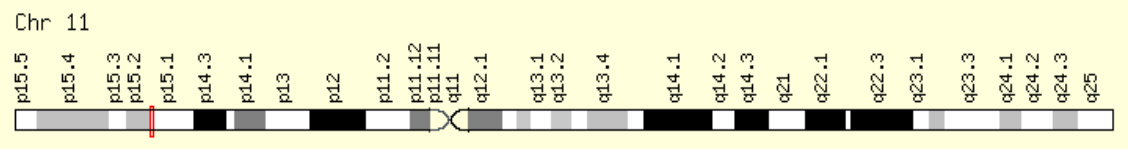

Figure1: location of the gene in chromosome 11 (11p15.2-p15.1)

The function of *PLEKHA7* was reported to be a component of the zonula adherens, a specialised cadherin-based cell–cell junction (Meng *et al.*, 2008). In humans, the gene is mapped to chromosome 11p15.1. *PLEKHA* gene has been associated with many diseases in relation to the eye, notably age related maculopathy and angle closure glaucoma (Connell *et al.*, 2009; Vithana *et al.*, 2012; Wei *et al.*, 2014).

1.4.2.2 *ABCC5*

ABCC5 (ATP-binding cassette, subfamily C, member 5), located in chromosome 3q27.1. The protein coded by this gene involved in multi-drug resistance, cellular and export mechanism (Wijnholds *et al.*, 2000). Studies speculated that this protein probably involved in resistance to thiopurines in acute lymphoblastic leukaemia and

antiretroviral nucleoside analogues in HIV-infected patients (Reid *et al.*, 2003). ABCC5 was also shown to have a significant association with PACG, as it has a strong contribution to anterior chamber depth (Nongpiur *et al.*, 2014).

1.4.2.3 *KALRN*

KALIRIN; KALRN is a multidomain guanine nucleotide exchange factor (GEF) for small GTP-binding proteins of the Rho family (McPherson *et al.*, 2002). McPherson also determined that the *KALRN* gene contains 60 coding exons and spans more than 600 kb. Intron 10 contains an internal start site from which the delta-kalirin transcripts initiate. *KALRN* gene at chromosome 3q13 in a fine-mapping study of the chromosome 3q region linked to early-onset coronary artery disease (Wang *et al.*, 2007). *KALRN* gene was found to involve in the Rho Guanosisne triphosphatase (GTPase) signal transduction pathway or also known as Rho-kinase (ROCK) pathway that influences cellular activity in angle structure (Somlyo *et al*., 2000; Wang *et al*., 2007). This pathway influences the function of trabecular meshwork. PACG is closely related to trabecular meshwork and angle structure changes. However, there was no available study assessing the role of *KALRN* gene in PACG.

1.5 RATIONALE OF THE STUDY

Glaucoma is the leading cause of a complex and irreversible blindness worldwide. It is a global phenomenon which is expected to increase in the future. The severity of bilateral blindness in PACG exceeds that of POAG with the majority of PACG found in Asia due to a higher prevalence among Asians. IOP remains the only modifiable

risk factors, hence in recent years; there is an increased interest to identify risk factors, pathophysiology and preventive measures to halt the progression of glaucoma. There are several susceptible genetic markers for PACG identified through GWAS. However, there was no fruitful finding of genetic markers for progression of PACG. To the best of our knowledge, there is no study that evaluates the relationship between PACG related SNPs like *ABCC5* and *PLEKHA7* with disease progression in Malay patients. *KALRN* gene was identified as susceptible genetic marker for progression of POAG in Malay patients. *KALRN* gene has been linked to ROCK pathway that influences cellular activity involving trabecular meshwork, aqueous humor drainage and retinal circulation. In fact, PACG is closely related to trabecular meshwork and angle structure activities. However, no study conducted involving *KALRN* gene and PACG.

Many studies regarding glaucoma have been conducted around Asia and other ethnicity, but studies on Malays, which represents 5% of the world population, is disappointedly lacking. The knowledge on genetics that affects the risk and progression of PACG may provide immense insight for possible primary intervention and future management of PACG. Genetics screening for markers associated with progression is important to high risk individuals; positive family history or with ocular risk factors for early intervention and prevention of blindness among glaucoma patients. Prevention of progression may also reduce the financial burden of treatment.

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