ASSOCIATION OF PDGFRA GENE POLYMORPHISM AND EARLY-ONSET MYOPIA IN SOUTH SUMATERA

HUBUNGAN ANTARA POLIMORFISME GEN PDGFRA DENGAN MIOPIA USIA DINI DI SUMATERA SELATAN

Thesis

Submitted to fulfill the assignment and fit-out requisite in passing Post-graduate Program

Master of Biomedical Sciences

Mitayani
22010112420044

FACULTY OF MEDICINE
DIPONEGORO UNIVERSITY
SEMARANG
2015
APPROVAL SHEET

Thesis

ASSOCIATION OF PDGFRA GENE POLYMORPHISM AND EARLY-ONSET MYOPIA IN SOUTH SUMATERA

By:

Mitayani
NIM: 22010112420044

Has been defended in front of the defense committee on 10th June, 2015 and has been approved by,

Principal Supervisor, Supervisor,

DR. dr. Tri Indah Winarni, M.Si Med Prof. DR. dr. Winarto, Sp.MK, Sp.M(K)
NIP.19660510 199702 2 001 NIP. 194906171978021001

Recognition,
Head of Master’s Degree Program in Biomedical Science
Faculty of Medicine Diponegoro University

dr. A. Zulfa Juniarto, M.Si Med, Sp.And, PhD
NIP. 197006081997021001
DECLARATION

I hereby declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education, there are no elements belonging Plagiarism forth in Decree No. 17 of 2010. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of reference is given.

Semarang, 06\textsuperscript{th} June, 2015

Mitayani
CURRICULUM VITAE

Name : Mitayani

Date and place of birth : Palembang, 01 Maret 1982

Address : Jl. Padang Selasa RT.17 No.4 Bukit Lama, Palembang

Religion : Islam

Status : Married

Email : mitayani.dr@gmail.com

Formal education :


4. SMU Xaverius 1 Palembang (1997-2000)

5. Faculty of Medicine Padjadjaran University (2000-2006)

6. Postgraduate Programme Faculty of Medicine Diponegoro University (2013-2015)

Working experience :

1. General practitioner at Amanah Medical Clinic, Sumedang (2006-2010)

2. Lecturer at Faculty of Medicine Muhammadiyah University, Palembang (2011-present)
PREFACE

One of the biggest eye diseases is nearsightedness (myopia). As a multifactorial disease, myopia can be caused by genetic or environmental factors. There are some risk factors for having early-onset myopia, such as sex, family history, corneal curvature, and genetic susceptibility. There is a need for information about these two risk factors that leads the author to write “Association of \textit{PDGFRA} gene polymorphism and early-onset myopia in South Sumatera”.

Biggest thanks to Allah SWT for the bless so this manuscript can be finished. Many thanks to DR. dr. Tri Indah Winarni, M.Si Med, Prof. DR. dr. Winarto, Sp.MK, Sp.M(K), Prof. Dr. Sultana M.H. Faradz, Ph.D, DR. dr. R.A. Kisdjamiatun R.M.D., M.Sc, Dean and staffs of Faculty of Medicine Muhammadiyah University Palembang, Director and staffs of Center for Biomedical Research Faculty of Medicine Diponegoro University for all supports and advices. Many thanks to my beloved husband and children, parents, and colleagues for all the love and supports.

Author realised that this manuscript is not perfect. But, author hope this manuscript can provides more information about genetics in Indonesia especially in ophthalmology.

Semarang, 17$^{th}$ June 2015

Author
TABLE OF CONTENTS

Cover................................................................................................................. i
Approval Sheet........................................................................................................ ii
Declaration............................................................................................................. iii
Curriculum Vitae................................................................................................... iv
Preface................................................................................................................... v
Table of Contents............................................................................................... vi
List of Tables.......................................................................................................... viii
List of Figures......................................................................................................... x
List of Attachments............................................................................................... xii
Abbreviation.......................................................................................................... xii
Glossary.................................................................................................................. x
Abstract............................................................................................................... xv
Abstrak................................................................................................................. xvii

CHAPTER I INTRODUCTION
1.1 Background................................................................................................. 1
1.2 Research Question....................................................................................... 3
1.2.1 General Research Question................................................................. 3
1.2.2 Specific Research Question................................................................. 3
1.3 Aim of Study............................................................................................... 3
1.3.1 General Aim.......................................................................................... 3
1.3.2 Specific Aims......................................................................................... 4
1.4 Research Benefits....................................................................................... 5
1.5 Research Originality................................................................................... 5

CHAPTER II LITERATURE REVIEW
2.1 Cornea......................................................................................................... 7
2.2 Myopia......................................................................................................... 9
2.2.1 Risk Factors in Myopia........................................................................ 9
2.3 PDGFRA Gene.......................................................................................... 11

CHAPTER III THEORETICAL AND CONCEPTUAL SCHEME
3.1 Theoretical Scheme................................................................................... 16
3.2 Conceptual Scheme................................................................................... 17
3.3 Hypothesis................................................................................................ 17

CHAPTER IV RESEARCH METHOD
4.1 Research Field............................................................................................ 18
4.2 Research Period and Location................................................................. 18
4.3 Research Design........................................................................................ 18
4.4 Material....................................................................................................... 18
4.4.1 Population............................................................................................. 18
4.4.2 Sampling............................................................................................... 18
LIST OF TABLES

Table 1. List of previous studies about PDGFRA gene polymorphism……. 6
Table 2. SNPs that are nearest to the PDGFRA gene............................... 15
Table 3. Location of SNPs in PDGFRA gene........................................ 21
Table 4. Distribution of sex and family history in myopia...................... 28
Table 5. Distribution of near work activities as risk factor of myopia....... 29
Table 6. Distribution of outdoor activity and lighting as a risk factor for myopia................................................................. 30
Table 7. Frequency table for specific question for myopic subject.......... 30
Table 8. Distribution of pedigree in case group................................... 31
Table 9. Distribution of myopia based on refractive power.................. 31
Table 10. Prevalence risk for sex as a risk factor for myopia.............. 32
Table 11. Prevalence risk for parental history of myopia as a risk factor for myopia................................................................. 33
Table 12. Prevalence risk for paternal grandparent history of myopia as a risk factor for myopia.......................................................... 33
Table 13. Prevalence risk for sibling history of myopia as a risk factor for myopia................................................................. 34
Table 14. Prevalence risk for reading and television viewing distance as a risk factor for myopia.......................................................... 35
Table 15. p value for risk factor in questionnaire................................. 36
Table 16. Corneal curvature prevalence.............................................. 37
Table 17. Corneal curvature in myopic and normal subjects............... 37
Table 18. p value for association between corneal curvature and myopia... 38
Table 19. Corneal curvature in male and female subjects.................... 38
Table 20. Distribution of corneal curvature in myopic subjects.......... 39
Table 21. Corneal curvature in myopic subjects................................. 39
Table 22. Allele distribution of rs7676985 G>A.................................. 42
Table 23. Allele frequency of rs7676985 G>A................................. 43
Table 24. Allele distribution of rs17084051 C>A......................................... 46
Table 25. Allele Frequency of rs17084051 C>A........................................... 46
Table 26. Allele distribution of rs7677751 C>T............................................ 49
Table 27. Allele Frequency of rs7677751 C>T............................................. 49
Table 28. Allele distribution for rs2307049 G>A........................................ 52
Table 29. Allele frequency of rs2307049 G>A.......................................... 53
Table 30. Allele distribution of rs7682912 T>G........................................... 55
Table 31. Allele Frequency of rs7682912 T>G............................................ 56
Table 32. Allele distribution of rs7660560 G>A........................................ 58
Table 33. Allele Frequency of rs7660560 G>A.......................................... 59
Table 34. Allele distribution of rs2114039 T>C........................................ 61
Table 35. Allele Frequency of rs2114039 T>C.......................................... 61
Table 36. Association between mutant allele of SNPs and eye status........ 62
Table 37. p value for mutant allele in SNPs and corneal curvature......... 63
Table 38. Comparison between Minor Allele Frequency in South Sumatera
and Singaporean population................................................................. 63
Table 39. Correlation between mutant allele in SNPs and pedigree,
progressivity, and refractive power.................................................... 64
LIST OF FIGURES

Figure 1. Corneal layers................................................................. 7
Figure 2. Myopic refractive error.................................................. 9
Figure 3. PDFGRA gene.............................................................. 12
Figure 4. Platelet-derived growth factor receptors....................... 13
Figure 5. Receptor tyrosine kinase activation............................... 14
Figure 6. Manual keratometer Takagi, Japan................................. 19
Figure 7. Electrophoresis result of rs7676985 ARMS PCR using primer set A................................................................. 41
Figure 8. Electrophoresis result of rs7676985 ARMS PCR using primer set C................................................................. 42
Figure 9. Electrophoresis result of rs17084051 ARMS PCR using primer set A................................................................. 44
Figure 10. Electrophoresis result of rs17084051 ARMS PCR using primer set C................................................................. 45
Figure 11. Electrophoresis result of rs7677751 ARMS PCR using primer set B................................................................. 48
Figure 12. Electrophoresis result of rs7677751 ARMS PCR using primer set C................................................................. 48
Figure 13. Electrophoresis result of rs2307049 ARMS PCR using primer set A................................................................. 51
Figure 14. Electrophoresis result of rs2307049 ARMS PCR using primer set B................................................................. 52
Figure 15. Electrophoresis result of rs7682912 ARMS PCR using primer set A................................................................. 54
Figure 16. Electrophoresis result of rs7682912 ARMS PCR using primer set B................................................................. 55
Figure 17. Electrophoresis result of rs7660560 ARMS PCR using primer set A................................................................. 57
Figure 18. Electrophoresis result of rs7660560 ARMS PCR using primer set B........................................................................................................................ 57

Figure 19. Electrophoresis result of rs2114039 ARMS PCR using primer set A........................................................................................................................ 60

Figure 20. Electrophoresis result of rs2114039 ARMS PCR using primer set B........................................................................................................................ 60
LIST OF ATTACHMENTS

Attachment 1. Statistical analysis of risk factors questionnaire.............. 81
Attachment 2. Statistical analysis of corneal curvature.......................... 99
Attachment 3. Statistical analysis PDGFRA gene polymorphism.......... 105
Attachment 4. Statistical analysis of pedigree, progressivity, and refractive power................................................................. 114
Attachment 5. ARMS PCR result......................................................... 129
Attachment 6. Questionnaire of myopia risk factors......................... 135
Attachment 7. Ethical clearance......................................................... 138
Attachment 8. Informed consent....................................................... 139
## ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARMS PCR</td>
<td>Amplification Refractory Mutation System Polymerase Chain Reaction</td>
</tr>
<tr>
<td>CD140A</td>
<td>Cluster Designation 140A, Clusters of Differentiation 140A</td>
</tr>
<tr>
<td>CEBIOR</td>
<td>Center of Biomedical Research</td>
</tr>
<tr>
<td>cM</td>
<td>CentiMorgan</td>
</tr>
<tr>
<td>COL1A1</td>
<td>Collagen, Type I, Alpha 1</td>
</tr>
<tr>
<td>COL2A1</td>
<td>Collagen, Type II, Alpha 1</td>
</tr>
<tr>
<td>D</td>
<td>Dioptric</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribose Nucleic Acid</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic Acid</td>
</tr>
<tr>
<td>FRAP1</td>
<td>FK506 binding protein 12-Rapamycin Associated Protein 1</td>
</tr>
<tr>
<td>GEPIS</td>
<td>Gene Expression Profiling In Silico</td>
</tr>
<tr>
<td>GWAS</td>
<td>Genome Wide Association Study</td>
</tr>
<tr>
<td>HRM</td>
<td>High Resolution Melting</td>
</tr>
<tr>
<td>HRMA</td>
<td>High Resolution Melting Analysis</td>
</tr>
<tr>
<td>MYP3</td>
<td>Myopia 3</td>
</tr>
<tr>
<td>MYP17</td>
<td>Myopia 17</td>
</tr>
<tr>
<td>NCBI</td>
<td>National Center for Biotechnology Information</td>
</tr>
<tr>
<td>NIH</td>
<td>National Institutes of Health</td>
</tr>
<tr>
<td>NLM</td>
<td>National Library of Medicine</td>
</tr>
<tr>
<td>PC</td>
<td>Personal Computer</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>PDGF</td>
<td>Platelet-derived Growth Factor</td>
</tr>
<tr>
<td>PDGFRA</td>
<td>Platelet-derived Growth Factor Receptor, Alpha polypeptide</td>
</tr>
<tr>
<td>PDGFR2</td>
<td>Platelet-derived Growth Factor Receptor 2</td>
</tr>
<tr>
<td>PI3</td>
<td>Phosphatidyl Inositol 3</td>
</tr>
<tr>
<td>qPCR</td>
<td>Qualitative Polymerase Chain Reaction</td>
</tr>
<tr>
<td>RTK</td>
<td>Receptor Tyrosine Kinase</td>
</tr>
</tbody>
</table>
SCORM : Singapore Cohort study of the Risk factors for Myopia
SiMES : Singapore Malay Eye Study
SINDI : Singapore Indian Eye Study
SNP : Single Nucleotide Polymorphism
SP2 : Singapore Prospective Study Program
SPSS : Statistical Package for the Social Sciences
WHO : World Health Organisation
GLOSSARY

ARMS PC : PCR technique that used a pair universal primer and 1 allele specific primer. This technique will produced 1 control band and 1 inner band specific for 1 allele.

Cataract : Partial or complete opacity on or in the lens or capsule of one or both eyes.

Collagen : The principle protein of connective tissue.

Conjunctivitis : Inflammation of conjunctiva.

Cornea : The transparent part of the eye that covers the iris and the pupil and allows light to enter the inside.

Corneal curvature : Radius of horizontal and vertical meridian of cornea.

Fibroblast : Connective tissue cells which secrete an extracellular matrix rich in collagen and other macromolecules.

GWAS : An analysis comparing the allele frequencies of all available polymorphic markers in unrelated patients with a specific symptom or disease condition, and those of healthy controls to identify markers associated with a specific disease or condition.

HRMA : A simple and fast post-PCR analysis method used for identifying genetic variation in nucleic acid sequences based on PCR melting curve techniques.

Hyperopia : A condition in which visual images come to a focus.
behind the retina of the eye and vision is better for distant than for near objects, called also farsightedness.

Keratitis : Inflammation of the cornea.

Keratoconus : A non-inflammatory, usually bilateral protrusion of the cornea, the apex being displaced downward and nasally.

Myopia : A condition in which the visual images come to a focus in front of the retina of the eye resulting in defective vision of distant objects--called also nearsightedness.

Polymorphism : A common variation in the sequence of DNA among individuals, occurring in more than 1% of the population.

Presbyopia : Defective accommodation and inability to focus for near vision because of loss of lens elasticity.

Receptor : A molecule inside or on the surface of a cell that binds to a specific substance and causes a specific physiologic effect in the cell.

SNP : Common variations that occur in human DNA at a frequency of 1 every 1,000 bases.

Steep cornea : Corneal refractive power of ≥ 48 diopters.
Association of *PDGFRA* Gene Polymorphisms and Early-onset Myopia in South Sumatera

**ABSTRACT**

**Background:** Myopia is a refractive error that may caused by corneal curvature (CC) anomaly. The purpose of this study was to identify platelet-derived growth factor receptor alpha (*PDGFRA*) gene and to find a correlation between sex, family history, near work, lighting, outdoor activity, CC, *PDGFRA* gene polymorphism, and early-onset myopia in South Sumatera tribes.

**Method:** Using a random sampling method, population-based, cross sectional study included 100 subjects aged 18-40 years from Palembang, South Sumatera, Indonesia. Visual acuity was measured by Snellen Chart and CC was measured by manual keratometer. DNA sample from buccal swab was investigated with Amplification Refractory Mutation System PCR and visualized in agarose gel.

**Result:** Females were 4.21 times more likely to have early-onset myopia than male. Subjects with parental, sibling, and paternal grandparent history of myopia were 6.64, 3.55 and 3.58 times more likely to have early-onset myopia, respectively. Reading ≥6 times/week, reading >2 hours/day and television viewing distance ≤60 cm were 2.95, 2.85 and 19.13 times more likely to have early-onset myopia, respectively. Median of corneal curvature for right eye was 7.73 (7.07-8.63) mm and left eye was 7.73 (7.04-8.69) mm. There were no difference between CC in myopic and normal subjects. Distribution of mutant allele in rs17084051, rs7677751, rs7682912, and rs2114039 were bigger in myopic subject compare to normal subject. Significant $p$ value for association between *PDGFRA* gene polymorphism and early-onset myopia was found only in rs17084051 ($p = 0.009$) and rs7677751 ($p = 0.001$).

**Conclusion:** Mutant type allele A of rs17084051 and mutant type allele T of rs7677751 *PDGFRA* gene polymorphism, female sex, parental history of myopia, paternal grandparent history of myopia, frequency of reading ≥6 times per week, duration of reading > 2 hours per day, and television viewing distance ≤60 cm are associated with early-onset myopia in South Sumatera tribes.

**Keywords:** myopia, early-onset myopia, polymorphism, SNP, PDGFRA, corneal curvature, myopia risk factor, Indonesia, South Sumatera
Hubungan Antara Polimorfisme Gen PDGFRA dan Miopia Usia Dini di Sumatera Selatan

ABSTRAK

Latar Belakang: Miopia adalah suatu kelainan refraksi yang dapat disebabkan oleh anomali kelengkungan kornea mata. Tujuan penelitian ini adalah untuk mengidentifikasi polimorfisme pada gen platelet-derived growth factor receptor alpha (PDGFRA) dan untuk mencari hubungan antara faktor risiko myopia, kelengkungan kornea, polimorfisme gen PDGFRA dengan miopia usia usia dini di Sumatera Selatan.


Hasil: Perempuan memiliki kemungkinan terkena miopia usia dini 4,21 kali lebih besar dibandingkan laki-laki. Subjek dengan orang tua, saudara kandung, serta kakek nenek dari pihak ayah dengan riwayat miopia memiliki kemungkinan 6,64 kali, 3,55 kali, dan 3,58 kali lebih besar menderita miopia usia dini dibandingkan yang tidak. Subjek yang membaca ≥6 kali/minggu dan >2 jam/hari kemungkinan menderita miopia usia dini 2.95 kali lebih besar daripada yang tidak. Subjek yang menonton televisi dengan jarak ≤60 cm memiliki kecenderungan menderita miopia usia dini 19,13 kali lebih besar dibandingkan yang tidak. Median nilai kelengkungan kornea pada mata kanan adalah 7,73 (7,07-8,63) mm dan mata kiri 7,73 (7,04-8,69) mm. Tidak ada perbedaan kelengkungan kornea antara subjek miopia dengan subjek normal. Distribusi alel mutan pada rs17084051, rs7677751, rs7682912, dan rs2114039 lebih besar pada kelompok miopia dibandingkan normal. Aasosiasi antara polimorfisme gen PDGFRA dan miopia usia dini hanya ditemukan pada rs17084051 ($p = 0.009$) dan rs7677751 ($p = 0.001$).

Kesimpulan: Alel mutan A pada rs17084051 dan alel mutan T pada rs7677751 polimorfisme gen PDGFRA, perempuan, riwayat orang tua dan saudara kandung dengan miop, riwayat kakek nenek dari pihak ayah dengan miopia, membaca ≥6 kali/minggu dan >2 jam/hari, menonton televisi dengan jarak ≤60 cm berasosiasi dengan miopia usia dini pada suku Sumatera Selatan, Indonesia.

Kata kunci: miopia, miopia usia dini, polimorfisme, SNP, PDGFRA, kelengkungan kornea, faktor risiko miopia, Indonesia, Sumatera Selatan.