

**MOLECULAR GENETIC ANALYSIS OF NON SYNDROMIC
RETINITIS PIGMENTOSA IN INDONESIA USING HIGH
RESOLUTION HOMOZYGOSITY MAPPING**

**ANALISIS GENETIK MOLEKULER RETINITIS PIGMENTOSA
NON SINDROMIK DI INDONESIA DENGAN *HIGH
RESOLUTION HOMOZYGOSITY MAPPING***



Thesis

**Submitted to fulfill the assignment and fit-out requisite
in passing Post-graduate Program Majoring Genetics Counseling
Diponegoro University Semarang as a joint degree
with Radboud University Nijmegen Medical Centre**

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Majoring Genetics Counseling
Diponegoro University Semarang
2011**

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RESOLUTION HOMOZYGOSITY MAPPING**

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DECLARATION

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgement is made in the text.

Semarang, December 2011

Galuh Dyah Nur Astuti

ACKNOWLEDGEMENTS

Many people have contributed their skills into this research, this work would have been impossible without their help and assistance. First of all, I give immeasurable thanks to Prof. dr. Sultana MH Faradz, PhD, my supervisor, for all her supports, suggestions, her concern and attention to me during this study.

I would like to express my deepest gratitude to my supervisor, Prof. Frans P. M Cremers PhD, for his guidance, endless encouragement and all of the opportunities that have been given to me during this research that was performed within one year in The Department of Human Genetics Radboud University Nijmegen Medical Centre (RUNMC) for the molecular genetic analysis part of the study. I gratefully thank dr. Kentar Arimadyo Sulakso, SpM, Msi. Med. for his guidance, helps, suggestions and supports from the very early stage of this research, especially in collecting Retinitis pigmentosa patients and his knowledge sharing regarding his previous research. I would like to give my deep gratitude to Rob Collin, PhD for his supervision, guidance and knowledge sharing.

I wish to thank Anna Siemiatkowska, PhD, my mentor for her supervision, advice, and guidance in the research technique and basic knowledge in molecular genetics, particularly in ophthalmogenetics. I convey a special acknowledgement to all of the Blindness Genetics Group members RUNMC (written alphabetically), Alejo Estrada, Ajmal Muhammad, Danielle Bosch, Ellen Blokland, Imran Khan, Marijke Zonneveld, Susanne Roosing and also Bjorn Bakker, Esin Ozturk, Frederieke Schoenmaker, Karin Littink, Krysta Voesenek, Mahesh Duvvari thank you for your kind help regarding my research, introducing me to the Dutch culture and for such a great friendship. To Prof. dr. Ben Hamel, PhD and Helger Ijntema, PhD for the wonderful opportunity that was given to me to study in RUNMC, The Netherlands.

I wish to also thank dr. Suwido Magnadi, SpM. and the Department of Ophthalmology Dr. Kariadi Hospital for the opportunity that was given to me to

had an internship in order to improve my knowledge and skill in the basic ophthalmic examination for RP patients.

To all the staff of Division of Human Genetics CEBIOR Faculty of Medicine Diponegoro University in Semarang Indonesia for your cooperation and friendship you have shared over the years. Particularly I would like to thank Wiwik Lestari, Lusi Suwarsi and Rita Indiarti for their time and technical finesse. To the staff of Central Java Eye Center for your kind help in the patients clinical examinations and patients data collection.

This work funded by Beasiswa Unggulan BPKLN Ministry of Education Republic Indonesia. My thanks to the staffs of Beasiswa Unggulan Project especially Dr. Abe Susanto and Dina Ardina, S.Sos from the staff of Biomedical Science Post Graduate Program. Thanks to Drs. Wil Groenen, Miranda Leenders, Ineke Zaalmlink and Mrs. Yayam Ruhuputty for their help in organizing my stay in Holland.

My gratitude to Rahajeng N. Tunjungputri for being a great reliable person to whom I could always talk about my problems and excitements. I would give my special thanks for my parents, my brother and friends that always give me an endless supports during this research.

Finally, I would like to thank all of the patients and their families that were participated in this research, this research would not be possible without your involvement and cooperation.

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ABBREVIATIONS LIST

11-cis-RAL	: 11-cis-retinaldehyde
11-cis-ROL	: 11-cis-retinol
<i>ABCA4</i>	: ATP-binding cassette, sub-family A (ABC1), member 4
adRP	: autosomal dominant retinitis pigmentosa
All-trans-ROL	: all trans retinol
ARMS	: amplification refractory mutation system
arRP	: autosomal recessive retinitis pigmentosa
<i>C2orf71</i>	: Chromosome 2 open reading frame 71 gene
cbEGF	: calcium binding Epidermal Growth Factor
cGMP	: cyclic Guanosine Monophosphate
<i>CLRN1</i>	: Clarin1 gene
<i>CRB1</i>	: Crumbs Homolog 1 gene
DNA	: Deoxyribonucleic acid
dNTP	: Deoxyribonucleotide triphosphate
EDTA	: Ethylenediaminetetraacetic acid
EGF	: Epidermal Growth Factor
GDP	: Guanosine diphosphate
GMP	: Guanosine monophosphate
GTP	: Guanosine triphosphate
IPM	: Interphotoreceptor matrix
<i>LCA5</i>	: Leber Congenital Amaurosis 5 gene

<i>LRAT</i>	: Lecithin Retinol Acid Transferase gene
NGS	: Next Generation Sequencing
PCR	: Polymerase Chain Reaction
PDE	: Phosphodiesterase
rAAV2	: Recombinant Adeno-associated Virus Vector
RFLP	: Restriction Fragment Length Polymorphism
RP	: Retinitis Pigmentosa
<i>RPI</i>	: Retinitis Pigmentosa 1 gene

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1. Association of in vitro antifungal activity test of buah merah (*Pandanus conoideus*) extract to the growth of *Candida albicans* in Candidiasis Vaginalis in collaboration with Department of Microbiology and Department of Derma-venereology. Paper for undergraduate research project (2006-2007)
2. Molecular genetic analysis of Retinitis Pigmentosa in Indonesian families using high resolution homozygosity mapping. Thesis for master studies of Biomedical Sciences Majoring Genetics Counseling Diponegoro University Semarang in collaboration with Department of Human Genetics Radboud University Nijmegen Medical Centre (2010-2011)

List of Publications

Siemiatkowska AM, Arimadyo K, Moruz LM, Astuti GDN, de Castro Miro M., Faradz S.M, Zonneveld-Vrieling MN, et al. Molecular genetic analysis of retinitis pigmentosa in Indonesia using genome-wide homozygosity mapping. Mol. Vis. 2011;17:3013-24.

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MOLECULAR GENETIC ANALYSIS OF NON SYNDROMIC RETINITIS PIGMENTOSA IN INDONESIA USING HIGH RESOLUTION HOMOZYGOSITY MAPPING

Backgrounds: Retinitis Pigmentosa (RP) is the most common inherited retinal diseases characterized by poor night vision, visual field constriction and central vision loss at later stage. Understanding on molecular genetics of RP and gene therapy approach has developed tremendously over the past few years. Nevertheless, the number of studies describing the molecular genetics related to RP in the Indonesian population is limited.

Objective: To describe the genetic defects responsible for non syndromic RP in Indonesia using high resolution homozygosity mapping

Methods: All affected individuals were clinically evaluated. Blood samples of all affected individuals and their family members were obtained. The DNA of all affected patients was analyzed for homozygous regions by Illumina 700K SNP array analysis followed by homozygosity mapping using PLINK software. Known RP genes residing in the identified homozygous regions were analyzed by direct Sanger DNA sequencing. Mutation confirmations were performed using segregation analysis and frequency analysis in ethnically matched healthy controls.

Results: In present study, three causative homozygous mutations have been identified in *CRB1*, *RPE65* and *RPI* genes.

Conclusions: This study revealed three novel mutations in *CRB1*, *RPI* and *RPE65* gene as genetic defects responsible for RP in Indonesian families. Mutations in *RPI* and *RPE65* genes are novel mutations, whereas the *CRB1* mutation has been described in the previous study in Indonesian population. The inheritance mode of the RP families is 17.9% and 82.1% are sporadic cases that are still remain unidentified.

Keywords: retinitis pigmentosa, high resolution homozygosity mapping, SNP array analysis, mutation

ANALISIS GENETIK MOLEKULER RETINITIS PIGMENTOSA NON SINDROMIK DI INDONESIA DENGAN *HIGH RESOLUTION HOMOZYGOSITY MAPPING*

Latar Belakang: Retinitis Pigmentosa (RP) merupakan penyebab terbanyak dari penyakit retina yang diturunkan. Penyakit ini ditandai dengan rabun senja, penyempitan luas lapangan pandang perifer diikuti penurunan tajam penglihatan pada lapangan pandang sentral. Genetik molekuler dan terapi genetik untuk RP berkembang sangat pesat beberapa tahun terakhir. Akan tetapi, penelitian molekuler genetik mengenai RP di populasi Indonesia masih sangat terbatas.

Tujuan: Untuk menjelaskan kelainan genetik yang terdapat pada penderita RP non sindromik di Indonesia dengan menggunakan *high resolution homozygosity mapping*

Metode: Pemeriksaan klinis dilakukan pada seluruh penderita RP yang bersedia mengikuti penelitian ini. Sampel darah dari penderita dan keluarga diambil untuk kemudian dilakukan ekstraksi DNA. Sampel penderita RP dianalisis menggunakan Illumina 700K SNP array, kemudian diikuti dengan homozygosity mapping menggunakan software PLINK. Gene RP yang sudah pernah diketahui sebelumnya, dipilih sebagai kandidat gene untuk kemudian dilakukan sequencing DNA. Konfirmasi mutasi yang ditemukan dilakukan dengan analisis segregasi dan analisis frekuensi pada kontrol sehat dari etnis yang sama.

Hasil: Pada penelitian ini, ditemukan tiga mutasi homozygous pada gen *CRB1*, *RPE65* and *RPI*.

Kesimpulan: Pada studi ini ditemukan tiga mutasi baru pada gen *CRB1*, *RPI* and *RPE65* sebagai penyebab RP di Indonesia. Mutasi di gen *RPI* dan *RPE65* yang ditemukan pada penelitian ini belum pernah dilaporkan pada penelitian lain sebelumnya. Pola penurunan RP pada studi ini adalah 17.9% autosomal resesif dan 82.1% kasus sporadik.

Kata kunci: retinitis pigmentosa, high resolution homozygosity mapping, analisis SNP array, mutasi