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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Biomedical Science Post Graduate Program
Majoring Genetics Counseling
Diponegoro University Semarang
2011

Thesis

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By
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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

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ABBREVIATIONS LIST

11-cis-RAL : 11-cis-retinaldehyde

11-cis-ROL : 11-cis-retinol

ABCA4 : 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

C2orf71 : Chromosome 2 open reading frame 71 gene

cbEGF : calcium binding Epidermal Growth Factor

cGMP : cyclic Guanosine Monophosphatase

CLRN1 : Clarin1 gene

CRB1 : Crumbs Homolog 1gene

DNA : Deoxyribonucleic acid

dNTP : Deoxyribonucleotide triphosphate

EDTA : Ethylenediaminetetraacetic acid

EGF : Epidermal Growth Factor

GDP : Guanosine diphosphatase

GMP : Guanosine monophosphatase

GTP : Guanosine triphosphatase

IPM : Interphotoreceptor matrix

LCA5 : Leber Congenital Amaurosis 5 gene

LRAT	: 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
RP1	: Retinitis Pigmentosa 1 gene

CURRICULUM VITAE

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Educations

• High School : SMU 3 Semarang (2000 - 2003)

• Bachelor degree : Faculty of Medicine Diponegoro University (2003 - 2007)

• Medical Degree : Faculty of Medicine Diponegoro University (2007 - 2009)

• Master Degree : Master Program of Biomedical Science Diponegoro University (2010 - present)

Scholarship

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Work experiences

1. Teaching and Laboratory Assistant of Medical Physics, Faculty of Medicine Diponegoro University (2004-2006)
2. Teaching and Laboratory Assistant of Clinical Parasitology, Faculty of Medicine Diponegoro University (2005-2006)
3. General Practitioner at Primary Health Centre, Welahan Jepara, Indonesia (2009)
4. General Practitioner at Mardi Mulya Clinic Semarang (2009 – 2010)
5. Academic staff at Department of Clinical Parasitology, Faculty of Medicine Diponegoro University (2009 – present)

Seminars and Courses

1. Medical Training, conducted by Faculty of Medicine Diponegoro University, Semarang, Indonesia (December 2006)
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3. Advanced Cardiac Life Support Training, conducted by Indonesian Heart Association, Semarang, Indonesia (November 2009)
4. Medical Genetic Course : From Basic to Clinic, conducted by Center for Biomedical Research (Cebior), Faculty of Medicine Diponegoro University in collaboration with Department of Human Genetics Radboud University Nijmegen Medical Centre, Semarang, Indonesia (January 2010)
5. Hematology-Medical Oncology Update : From Bench to Bed, conducted by Internal Medicine Department of Dr. Kariadi Hospital in collaboration with Radboud University Nijmegen Medical Centre, Semarang, Indonesia (January 2010)
6. 2nd International Seminar and Workshop on Fragile-X Autism and Related Disorders, conducted by Center for Biomedical Research (Cebior), Faculty of Medicine Diponegoro University in collaboration with Medical Investigation of Neurodevelopmental Disorders (MIND) Institute UC Davis, Semarang, Indonesia (August 2010)
7. European Human Genetics Conference 2011, conducted by European Society of Human Genetics, Amsterdam, The Netherlands (May 2011)

8. 4th World Glaucoma Congress, conducted by The World Glaucoma Association, Paris, France (June - July 2011)
9. 2nd Course in Eye Genetics, conducted by European Genetics Foundations, Bologna, Italy (September - October 2011)

Research Experiences

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.

Membership in Professional Society

Indonesian Medical Association (2010 – Present)

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 contriction 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 *RP1* genes.
Conclusions: This study revealed three novel mutations in *CRB1, RP1* and *RPE65* gene as genetic defects responsible for RP in Indonesian families. Mutations in *RP1* 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 molekular 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 suquencing DNA. Konfirmasi mutasi yang ditemukan dilakukan dengan analisis segregasi dan analisis frekuensi pada kontrol sehat dari etnis yang sama.
Hasil: Pada peneltian ini, ditemukan tiga mutasi homozygous pada gen *CRB1*, *RPE65* and *RP1*.
Kesimpulan: Pada studi ini ditemukan tiga mutasi baru pada gen *CRB1, RP1* and *RPE65* sebagai penyebab RP di Indonesia. Mutasi di gen *RP1* 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